

STUDIES ON FACTORS AFFECTING DISSOLVED  
OXYGEN CONCENTRATIONS IN AEROBIC  
OXIDATION PONDS

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OXYGEN CONCENTRATIONS IN AEROBIC  
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## CHAPTER I

### INTRODUCTION

Reuse of water resources is recognized as the only method which, in the future, will satisfy the ever-increasing demand for the indispensable necessity, water. Therefore, it is natural that the removal of organic matter, soluble and colloidal, will receive increasing attention. The oxidation pond is one of the widely employed secondary treatment processes. It is also often employed as the sole treatment process in some places, and as a tertiary process for polishing the secondary effluent in other cases. Unlike other secondary processes, there are not many modifications of the basic process. The study of factors governing the purification process in such ponds provides challenging opportunities to improve upon the existing process and, possibly, to develop new modifications.

Even though the impoundment of waste water was practiced long ago, the purification powers of oxidation ponds were accidentally discovered at Santa Rosa, California, in 1924 (1). In 1929, an oxidation pond was designed and constructed for the town of Sonoma, California. In 1933, Texas A. & M. College constructed a 14-acre lake. It gave satisfactory results in spite of the high BOD loading of

475 ppm (2). The city of Imperial switched to this system in 1941, followed by the city of Modesto, and many others. At present this process is used throughout the world. In a survey conducted in 1959 it was found that 652 cities in the United States were employing this process (3). Porges reported in 1963 that 827 ponds were used for treating industrial wastes from twenty different industries (4).

A brief analysis of stabilization pond literature (which included seventy-nine references) was made by Fitzgerald and Rohlich in 1958 (5). Yeoh evaluated the oxidation pond as a waste treatment process in 1965 (6). His report contained eighty-three references. Both reports concluded that within certain limitations this process will yield good results.

Despite the fact that this process is widely employed, procedures for design of oxidation ponds are far from satisfactory and, for the most part, they are designed either on an empirical basis or on a "similar type" or precedent design basis. A few significant attempts have been made (7) (8) (9) (10) (11) to rationalize the design procedures. In a recent publication (11) an attempt was made to develop design criteria based upon reactor design in the chemical engineering field. In the author's opinion, none of the above has solved the problem completely. One of the reasons for the failure to arrive at widely acceptable and practicable rational design criteria is oversimplification of the various interdependent processes. Many intricate factors

are not taken into consideration, and are sidestepped. Under these circumstances any attempt to rationalize the design criteria on a scientific basis is bound to fail. Understanding the functions of the various interconnected reactions and measurement of the magnitude and the rate of the constituent processes are prerequisites for the development of any sound formulae for oxidation pond design.

An engineer is one who "measures and controls." In the preceding paragraph the importance of "measurement" was indicated. Once the effect of a particular factor on the composite system can be measured satisfactorily, steps should be taken to control that factor with the ultimate objective of improving the efficiency of the composite system. Two such factors which are important in oxidation ponds are light intensity and carbon-dioxide concentration.

Within certain limits, the greater the light intensity, the faster will be the algal growth rate, with concomitant production of more oxygen (12)(13). The excessive oxygen produced will help to keep the oxidation pond aerobic. Even in places where light intensity is excessive during a normal day (e.g., Oklahoma), light may be growth-limiting on cloudy days. To offset this and to utilize the excessive light energy more effectively, Mayer, et al. (14) attempted to introduce light into the depths of mass cultures. They used inverted transparent pyramids, thereby essentially increasing the lighted surface. Such "controls" are not yet practiced in waste water purification.

Carbon dioxide, regardless of its origin, is the carbon source for algal growth under photo-autotrophic conditions. Growth increases as the  $\text{CO}_2$  concentration in the medium increases. Since the partial pressure of  $\text{CO}_2$  in the atmosphere is very low, the  $\text{CO}_2$  concentration in any body of water equilibrating with the atmosphere (e.g., an oxidation pond) will also be low. When the  $\text{CO}_2$  produced by bacterial metabolism exceeds the saturation concentration in the medium, it tends to be stripped to the atmosphere. If stripping could be prevented by means of an impermeable sheet, an "accelerated symbiosis" might be forced. The increased  $\text{CO}_2$  concentration would be of help in accelerating algal growth which, in turn, might lead to increased purification efficiency. Innovations such as this have not been reported to date, and such study could lead to improvements upon the existing oxidation pond process.

The objective of the present research was to study the significant processes causing a change in the dissolved oxygen (DO) concentration in oxidation ponds. To accomplish this objective, biological deoxygenation, by both bacteria and algae, was investigated separately. The physical reaeration and deaeration characteristics of the experimental ponds were studied. Furthermore, studies in open ponds and corresponding studies in ponds closed to the atmosphere were undertaken in order to aid in the assessment of the effect of free gas transfer on changes in DO concentration when biological deoxygenation and physical aeration were

occurring simultaneously under various organic loadings.

It was felt that separate investigation of each major process affecting DO concentration could provide useful information pertaining to the critical factors for maintenance of aerobic conditions in oxidation ponds. For example, it was envisioned that the work would allow definite conclusions to be drawn concerning the relative contributions to the DO resource of the system due to algae and to physical transfer of  $O_2$  across the air-liquid interface of the pond.

## CHAPTER II

### LITERATURE REVIEW

#### General Principles

Figure 1 is a simplified schematic diagram of the various inherent constituent processes in, and components of, an aerobic oxidation pond. The organic wastes are degraded by bacterial action. Dissolved oxygen (DO) in the medium is utilized for this oxidative process. The significant end products of this process are carbon dioxide and water. When light energy along with inorganic nutrients are present, and in the absence of inhibitory substances, the carbon dioxide is used by algae as a carbon source. Oxygen is evolved as a byproduct in this process of photosynthesis. This oxygen replenishes the DO level depleted by bacterial metabolism, and thus the cycle is completed.

Some portion of the organic wastes may be metabolized (heterotrophically) by algae to  $\text{CO}_2$  at the expense of the DO resource. Alkalinity present in the waste may also contribute to the  $\text{CO}_2$  pool. The  $\text{CO}_2$  and  $\text{O}_2$  in the medium are always subject to transfer to the atmosphere, dependent upon their concentration in the medium. From the above considerations it can be seen that (1) organic wastes,

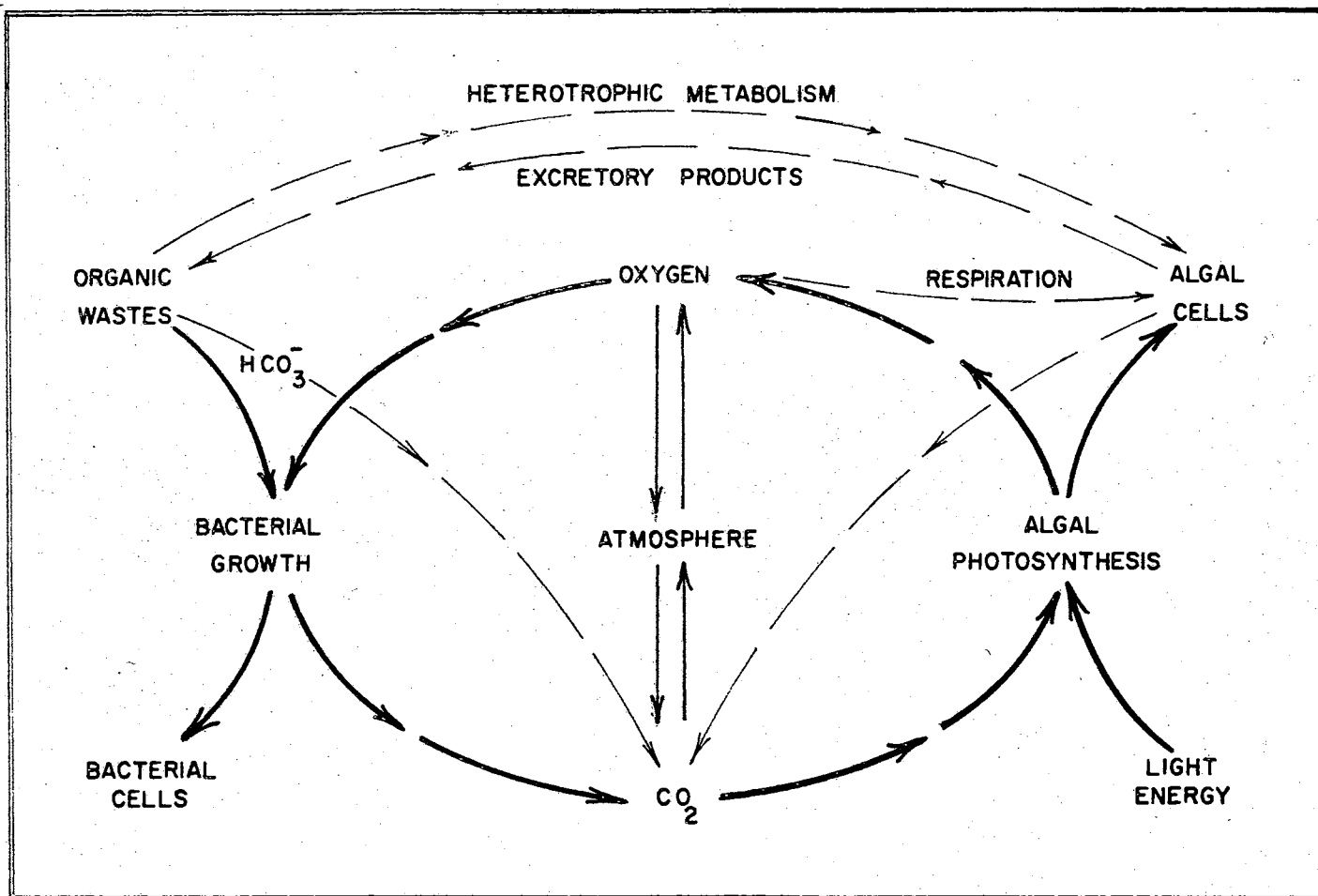


Figure 1 - The cycle of constituent processes in an aerobic oxidation pond.



(2) bacterial oxidation, (3) carbon dioxide, (4) light energy, (5) algal photosynthesis, and (6) dissolved oxygen are paramount factors in the functioning of aerobic oxidation ponds.

### Photosynthesis

The fact that the photosynthetic reactions consist of two distinct phases is well known, and detailed information can be found in many texts (15)(16). The "light" reactions are energetic in nature. The electromagnetic radiation energy of the light is absorbed by the photosensitive systems containing the chlorophylls, and this light energy is used to bring about the transfer of electrons from the water molecule. This results in the formation of TPNH and ATP with concomitant evolution of  $O_2$ . In the "dark" reactions, the available carbon dioxide is reduced to the level of sugar phosphates, at the expense of the co-factors formed in the light reactions.

### Light

From the above it can be discerned that light intensity exerts a controlling effect on the rate of photosynthesis through the "light" reaction. However, once the rate of the light reaction is equal to the rate-limiting step of the dark reactions, an increase in the light energy will not increase the overall rate of photosynthesis; therefore, it is reasonable to expect that the photosynthetic rate depends upon the intensity of light only within certain limits. The upper limit is imposed by the rate of

the dark reaction which, in turn, depends upon the species present, available substrate, and other environmental factors. The lower limit, the so-called compensation point, depends upon the species present and their physiological condition. At this light intensity, all of the oxygen produced is required to meet the respiratory demands.

Rodhe attempted to elucidate the influence of some environmental factors on the development of fresh water plankton algae (17). He conducted experiments with Ankistrodesmus falactus under different temperatures (15, 20, and 25°C) and light intensities (1000, 1700, and 3600 lux). The cultures were illuminated ten hours per day. The results indicated higher growth at 3600 lux (about 360 foot candles [ft-c]) at all three temperatures. However, he cautioned that the individual organisms probably do not have fixed temperature and light optima, but that their levels may be also dependent on the periodicity of the lighting.

Varma and Wilcomb published a paper entitled "Effect of Light Intensity on Photosynthesis" (18). They reported that they have made metabolic studies of algae using a Warburg respirometer and BOD bottles. They did not state what type of algae they used, or the source of algae. However, in a previous paper (19) Varma, et al. indicated that Oscillatoria was the predominating alga in their system. BOD bottles were incubated at 30°C under continuous lighting. The light intensities varied from 260 to 1200 ft-c.

The experiments in the Warburg apparatus were conducted at 37°C and at light intensities of 816 and 1450 ft-c. Though many essential details are missing, the reported results show that oxygen was evolved at increasing rates with increased intensities of light.

Lubbers and Parikh (20) studied the effect of light intensity and other factors upon growth of mixed algal cultures from experimental waste stabilization lagoons. They used raw, homogenized sewage having a 5-day BOD value of 350 to 650 mg/l. The "algal culture" would be expected to contain other microbes as well, since the culture was taken from experimental lagoons. The light intensity was varied from 10 ft-c to over 9000 ft-c. They reported the compensation point (light intensity when net oxygen production is zero) as slightly less than 20 ft-c. They stated that 720 ft-c "may be considered as a saturation value." The reason for this indefinite statement may be the design of the experiments. The experiments were conducted at about 10, 20, 40, 70, 140, 300, 720, 1000, 3300, 4500 ft-c, etc. The net O<sub>2</sub> production increased until 720 ft-c was reached; above this value it remained almost constant. They could have made a definite statement that at 720 ft-c (as at 1000 and 3300 ft-c) O<sub>2</sub> production was maximum. The saturation point was somewhere between 300 and 720 ft-c. From their arithmetic plot of the data one would estimate the value to be around 550 ft-c. This report would have been more useful had the authors indicated the concentration of the

culture in these particular experiments.

Oswald, et al. (12) experimented with Euglena gracilis, employing natural and synthetic sewage in semicontinuous flow units. The BOD of the natural sewage was 90 mg/l, and that of the synthetic sewage was 117 mg/l. The retention periods employed were seven and five days, respectively, and the range of light intensity was 100 to 2400 ft-c. They reported the value of the saturation point to be at or near 400 ft-c for the low BOD (natural sewage) system run at a detention time of seven days. However, on the synthetic sewage, the growth increased until the 2400 ft-c level was attained. Even with this high light intensity, growth was lower than the growth at 400 ft-c on the natural sewage system. They concluded that the optimum light intensity depends on retention time and strength of sewage. It is possible that their synthetic sewage was lacking or low in some essential growth nutrient.

Myers (13) (21) in 1946 presented data relating growth rate to light intensity. Chlorella pyrenoidosa was grown in a continuous culture, the intensity of light being varied from 6 ft-c to 360 ft-c in one series, and from 9 ft-c to 325 ft-c in another. Both sets of experiments indicated a levelling off of the growth curve at approximately 100 ft-c. He concluded that "at low intensities (<60 ft-c) growth is proportional to light intensity. At high intensities (>100 ft-c) growth is nearly independent of light intensity." Seven years later, the same author determined the light

intensity curve for growth of Chlorella pyrenoidosa in connection with the determination of growth rate in flashing light (22). In this work he estimated the value of the compensation point to be less than 24 ft-c. He stated that the growth rate does not have a light saturation, but continues to increase slowly with increasing intensity. His estimate of the "saturation value," wherein the growth rate attained ninety per cent of its observed maximum, was 600 ft-c. He accounted for this discrepancy in the saturation values by the difference in the nature of the illumination. The value of 100 ft-c was arrived at when the light was multilateral (from many directions) and the value of 600 ft-c was obtained when the illumination was unilateral (from the top only). The difference, to a certain extent, may be due also to changes in the lighting system and modification of the medium in the two studies.

In a book edited by Burlew, Myers discussed (23) the growth characteristics of algae in mass culture. He stated that "the minimum intensity required for maximum rate of growth of Chlorella is in the neighborhood of 400 ft-c under unilateral illumination."

Considering the above discussion on the effect of the intensity of light, it can be surmised that under normal conditions encountered in oxidation ponds (at a temperature of approximately 25°C), 400 ft-c is close to "saturation value."

## Carbon Dioxide

One of the "driving forces" of the "dark reactions" is the carbon dioxide present in the medium. The incorporation of  $\text{CO}_2$  can take place during the light period also. The nature of the initial carboxylation reaction and the regeneration of the  $\text{CO}_2$ -acceptor, ribulose diphosphate, were unraveled primarily by the experiments of Calvin and co-workers (24). The metabolic pathway proposed by them for the photosynthetic carbon cycle is widely, although not universally, accepted (25).

Since  $\text{CO}_2$  is the substrate for the initiating step of the photosynthetic carbon cycle, the rate of the dark reactions depends on the  $\text{CO}_2$  concentration, within certain limits. Much conflicting information is available regarding these limits. Emerson and Green studied the rate of photosynthesis (measured by the rate of  $\text{O}_2$  evolution) as a function of  $\text{CO}_2$  concentration in the medium using Chlorella pyrenoidosa (26). Their results showed that the photosynthetic rate varied linearly with  $\text{CO}_2$  concentration up to approximately 0.05 per cent, and thereafter remained constant up to the maximum tested concentration of 5 per cent.

Spoehr and Milner (27) compared the effect of bubbling  $\text{CO}_2$  at concentrations of 3, 5, and 10 per cent (in the aerating gas) on the yield of Chlorella pyrenoidosa under two light intensities. At low intensity the yields in three and five per cent showed no significant difference,

while at the higher intensity the yields were roughly proportional to the  $\text{CO}_2$  concentration. In the culture grown with ten per cent  $\text{CO}_2$ , the yield was less, amounting to about eighty per cent of the five per cent  $\text{CO}_2$  culture. Despite the fact that measurement of  $\text{CO}_2$  concentration is different in the two cases referred to above, the saturation limits are far apart.

Davis, et al. (28), in an effort to ascertain the effect of  $\text{CO}_2$  on the growth rate of Chlorella, conducted experiments at an incident light intensity of 350 ft-c. The concentrations of carbon dioxide dissolved in the culture medium in these experiments were 4.43, 2.19, 1.02, and 0.56 per cent. Within experimental variations, the growth rates were the same. They stated that with low concentrations of carbon dioxide, the gas must be supplied fast enough so that the culture medium is in equilibrium with the  $\text{CO}_2$  concentration in the gas stream.

Gaucher, et al., working with thermophilic Chlorella pyrenoidosa, attempted to control the photosynthetic rate by varying carbon dioxide and some other factors as the principal parameters (29). They used 0.5, 3.0, and 5.5 per cent  $\text{CO}_2$  in the aerating gas, and concluded that concentrations of  $\text{CO}_2$  around 0.5 per cent do not appear to limit the growth of the organism.

Even though the atmospheric air contains an abundant quantity of  $\text{CO}_2$ , its concentration is only 0.03 per cent. This is far below the "saturation value." Therefore, the

growth of algae in a body of water which is in continuous contact with the atmosphere, e.g., an open oxidation pond, may be limited by  $\text{CO}_2$  concentration. Field and laboratory data support this contention (30) (31) (32) (33) by pointing out that in many instances carbon is the growth-limiting factor.

Ludwig, et al. studied the growth characteristics of Euglena gracilis in sterilized sewage which was reseeded with sewage (30). They conducted three series of experiments using air with 2.30, 1.75, and 0.03 per cent  $\text{CO}_2$ . The maximum algal yields were 0.23, 0.22, and 0.08 grams/liter/day, showing a linear relation in a semi-log plot. In a similar study with Chlorella pyrenoidosa (31), analyses were made on algal cells and the sewage medium. Calculations showed that the sewage provides all nutrients in excess except carbon. Allen investigated algal growth in sewage oxidation ponds in California (32). She isolated a strain of Chlorella, CC-2, from a pond in Contra Costa, and studied its growth in sterilized sewage with and without  $\text{CO}_2$ . The concentration of  $\text{CO}_2$  was five per cent. After seven days, the growth was approximately three times higher in the culture with  $\text{CO}_2$ .

All of these studies have shown how algal growth rate can be increased by provision of additional  $\text{CO}_2$ . However, the increased algal growth, per se, will not improve the efficiency of waste water purification. In the oxidation pond, bacteria are mainly responsible for the oxidation of

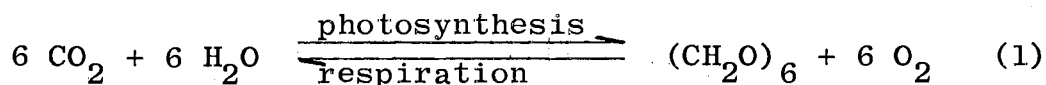


organic matter. In fact, during algal growth some organic materials are excreted into the medium (to be dealt with in some detail later) which will increase the pollutional load on the system. The prime function of algae in the ponds is to act as "biological aerators."

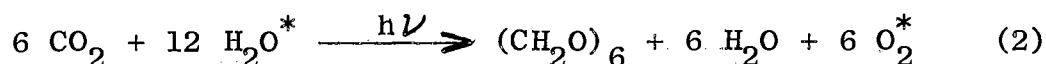
The effect of CO<sub>2</sub> availability on oxygen production can be seen from the following example. Hannan and Patouillet used a high temperature strain of Chlorella pyrenoidosa to relate CO<sub>2</sub> supplied with the oxygen produced (33). The input rate was adjusted by changing either the volume of flow or the concentration of gas. The CO<sub>2</sub> input was varied from 2310 to 3820 cc/hr. In four experiments out of five the oxygen produced could be correlated to the CO<sub>2</sub> supplied; furthermore, the density of the steady-state suspension could also be correlated to oxygen produced.

#### Photosynthetic Oxygenation

The classical definition of photosynthesis is usually given as "the reduction of carbon dioxide to carbohydrate." For convenience it is usually explained as the "reversal of respiration," and is denoted by the equation:



Since the source of the evolved oxygen has been found to be water, a more appropriate equation to describe photosynthesis is:



In accordance with this, 6 moles of  $\text{CO}_2$  are assimilated for the synthesis of one mole of carbohydrate with the simultaneous liberation of 6 moles of  $\text{O}_2$ .

Estimation of the oxygen evolved can be made directly by quantitative determination of the liberated  $\text{O}_2$ ; the Warburg apparatus has been used in such determinations (30) (31) (34). Extrapolation of the Warburg results to natural conditions may be of questionable validity. Apart from that, another unsolved and inherent difficulty is the effect of lighting on the normal respiration of cells. Hence, the determination by Warburg respirometer is the net oxygen production over and above the respiratory requirements, if any, under a different environment.

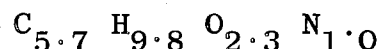
Another approach is the calculation of  $\text{O}_2$  evolved using the balanced photosynthetic equation, i.e., Equation (2). Assuming that the equation is correct, it can be seen that 6 moles of oxygen are evolved for 6 moles of  $\text{CO}_2$  consumed or for each mole of carbohydrate synthesized. Measurement of  $\text{CO}_2$  utilized for photosynthesis in purely photoautotrophic systems can be done with considerable accuracy. But it is not possible in heterotrophic systems or even when  $\text{CO}_2$  is fixed non-photosynthetically. The other alternative is the determination of the quantity of organic material synthesized and multiplication of this quantity by an appropriate factor.

There are two schools of thought concerning the multiplication factor. One is the "unit process" concept (35)

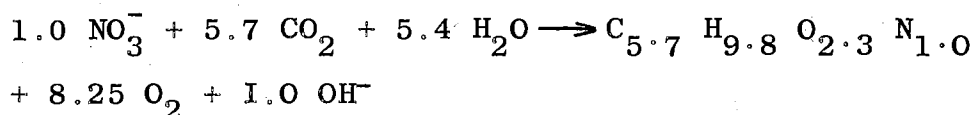
maintaining that the primary end product of photosynthesis is a hexose sugar even though the "first sugar" was not identified. This school of thought does take into account the fact that algal cells or, for that matter, any plant cells, are not composed of carbohydrate alone but contain other components as well. However, in accordance with this concept, nitrogen assimilation and other metabolic processes take place outside the photosynthetic cycle. If this is correct, the multiplication factor will be 6, and the assimilatory quotient ( $\text{CO}_2/\text{O}_2$  ratio) will be 1.0.

The other line of thought is the "meshed process" in which nitrogen assimilation and other metabolic processes are considered to be intimately related to photosynthesis. A quotation from Myers, who advocated this second possibility, is given below (34):

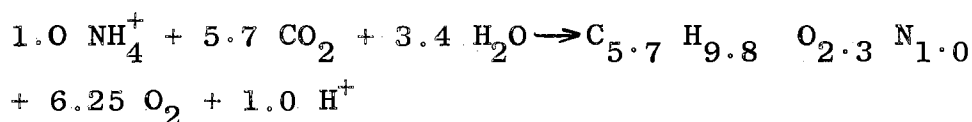
"Elementary analysis on Chlorella, as grown in our experiments, yields 53.0 per cent C, 7.5 per cent H, 28.5 per cent O, 10.8 per cent N, on an ash-free, dry weight basis. On dividing by the appropriate atomic weights, these percentages can be converted to the expression



If the nitrogen source is known, it becomes possible to write balanced equations for overall metabolism and thus predict the gas exchange. For instance:



$$\text{Quotient } \text{CO}_2/\text{O}_2 = - 5.7/8.25 = - 0.69$$



$$\text{Quotient } \text{CO}_2/\text{O}_2 = - 5.7/6.25 = -0.91."$$

It can be seen that the multiplication factor varies, depending not only upon the elemental composition of algal cells, but also on the nitrogen source.

Whether nitrogen assimilation takes place in the photosynthetic step may not be of much significance to the overall oxygen balance of oxidation ponds. The anticipated function of algae in the ponds is the provision of oxygen over and above their own respiratory needs. As such, the quotients estimated by considering actual composition and nitrogen source will be more appropriate. Oswald, et al. (12) changed over to this line of thought in the middle of their published series of reports (12) (30) (31). Taking ammonia as the nitrogen source for their Euglena cultures, they framed the equations and calculated assimilatory quotients and thereby the gross  $O_2$  production for light intensities from 100 to 2400 ft-c. Maximum  $O_2$  was produced at approximately 700 ftc and greater than ninety-three per cent of the maximum production was evolved at light intensities between 400 and 1200 ft-c. Furthermore, when they developed design criteria (8), they related the weight of oxygen evolved to the weight of cell material synthesized using the equation at 400 ft-c. They found 1.6 grams of  $O_2$  were produced for each gram of new cell material, and that this value may vary from 1.5 to 2.0.

Another method which is widely used in field studies is the light and dark technique. Several airtight bottles containing the culture are suspended at desired depths.

One-half of the number of bottles (dark bottles) are wrapped with light-excluding material. The difference between the DO changes in the two groups is the net oxygen production. In this method it is assumed that the respiration is identical in the light and in the dark. Bartsch and Allum (36) presented field observations of two oxidation ponds. At one foot below the surface, where the light intensity was about 1000 ft-c, the net production was six times the DO required for respiration. At a depth of two and one-half feet the light intensity was 167 ft-c and the net production was zero. At this point the DO consumed equalled the DO produced.

O'Connell and Thomas (37) studied the effect of benthic algae on dissolved oxygen in the Truckee River in Nevada. They evaluated the net oxygen contribution by two separate methods, and arrived at approximately the same values. In one method they used a modified sag equation containing a term for photosynthesis, evaluated all other terms, and thus determined the amount of photosynthesis. In the second method they measured the net oxygen change using airtight plexiglass-polyethylene sheet chambers of 10-liter capacity, which were immersed in the stream and from which samples could be withdrawn periodically. Oscillatoria, which was predominant in the reach of stream under study, was cultured in petri dishes. The petri dish, entirely covered with the alga, was placed in the chamber and flushed with river water. Test results were expressed

as mg of  $O_2$  per hour per unit area of alga. Along with this measurement, the calculation of the bottom area covered by the alga and the water volume in each reach enabled them to express the results in units of mg/liter/hr. The results showed that the maximum photosynthetic oxygen production occurred at approximately one o'clock in the afternoon. At one and one-half hours after sunrise and one and one-half hours before sunset, the respiratory consumption equalled the photosynthetic production. On the average, 72.5 lb. of  $O_2$ /acre/day was produced against the average respiratory demand of 65.4 lb. of  $O_2$ /acre/day.

#### Heterotrophy

The ability of algae to grow in darkness utilizing an organic carbon source was known even in the last century. However, the species that have this heterotrophic capacity, the substrates they can oxidize, and the mechanisms of this metabolism are scantily categorized (38). A few selected works which have a bearing on this research are summarized in Table I.

Pearsall and Bengry (39) cultured a strain of Chlorella in the dark, using a medium containing 10 g/l of glucose. They found that the growth, at first, was exponential in character until the cell number reached approximately 6000 cells per cubic millimeter. The logarithmic growth rate in the dark was about sixty per cent of the logarithmic growth rate in the light. After this, the growth curve was nearly linear in nature, which may have been due

TABLE I  
HETEROTROPHIC METABOLISM OF ALGAE

#	Authors	Ref.	Algae	Substrate	Results
1	Pearsall and Bengry	39	<u>Chlorella</u>	Glucose	Retarded, yet positive growth; exponential followed by linear in nature
2	Samejima and Myers	40	<u>Chlorella pyrenoidosa</u> <u>Chlorella elliposoidea</u> <u>Scenedesmus</u> sp. D3	Eleven sugars, three alcohols, sugar phosphates, and some fatty acids	The two chlorellae grew well only on glucose and galactose. The growth of <u>Scenedesmus</u> was good on glucose and slow on galactose, mannose, fructose, maltose, and lactose
3	Theriault	41	<u>Chlorella pyrenoidosa</u> 7-11-05	Single carbon source glucose, glycerol, galactose, sucrose, mannose, mannitol  Two carbon source glucose + <ul style="list-style-type: none">glycerol ) galactose ) fructose ) xylose ) arabinose )</ul>	Glucose was used well; glycerol 52% of glucose. Galactose 25% of glucose. Others not appreciable  Fair Good Toxic
4	Lewin	42	<u>Chlamydomonas dysosmas</u> 1. Wild type 2. Mutant	Acetate, lactate, pyruvate Succinate, malate, citrate, glutamate, glycerol, glucose, G-1-P	Wild type grew well in acetate, slightly in lactose or pyruvate, and no growth in others, including glucose. Mutant, no growth
5	Parker, et al.	43	<u>Chlorococcum</u> <u>Bracteacoccus</u> <u>Spongiochloris</u> <u>Dictyochloris</u>	Glucose, acetate	<u>Bracteacoccus</u> , <u>Spongiochloris</u> , and <u>Dictyochloris</u> grew; <u>Chlorococcum</u> did not grow
6	Finkle, et al.	44	<u>Chlorella vulgaris</u> (Emerson strain)	Glucose	No growth
7	Karlander and Krauss	45	<u>Chlorella vulgaris</u>	Glucose	No growth
8	Myers	46	<u>Chlorella vulgaris</u>	Glucose and peptone	Production of pigments
9	Allen	32	<u>Chlorella</u> <u>Scenedesmus</u> , etc.	Sewage	No growth
10	Pipes and Gotaas	47	<u>Chlorella pyrenoidosa</u>	Sewage	Cultures having detention period less than three days were able to utilize some of the organic matter
11	Pipes	48	<u>Chlorella pyrenoidosa</u>	Glucose, fatty acids with less than six carbons, five amino acids, urea  Sewage fractions soluble in (1) water, (2) acid, (3) hydroxide, (4) carbonate. Insoluble, soap-like amorphous precipitate	Growth rate was increased compared to the growth rate in inorganic medium. Water soluble sewage fraction increased growth

to the limitation of oxygen supply.

Samejima and Myers (40) studied the heterotrophic growth of Chlorella pyrenoidosa and two other organisms on various organic substrates. One per cent (weight/volume) substrate concentrations were employed. The specific logarithmic growth rate of Chlorella pyrenoidosa on glucose in the absence of light was almost one-half of its specific logarithmic growth rate in the light (0.46/day against 0.93/day). The growth rate on galactose was one-half the rate on glucose. When this strain was grown on a glucose and galactose medium, the growth rates were identical to the growth rates in medium containing only glucose, indicating a common rate-limiting reaction. If the cells have used glucose in preference to galactose (sequential substrate removal), that would explain the above observation. This could have been verified if the specific substrate removals had been followed.

Theriault (41) investigated the production of xanthophylls by Chlorella pyrenoidosa 7-11-05, grown heterotrophically. Glucose was the only sugar among the eleven sugars used as sole carbon source which gave appreciable growth. Glycerol and galactose utilization were approximately fifty-two and twenty-three per cent of glucose utilization. Fructose, which was not consumed when used as a sole carbon source, was almost completely assimilated when it was present in combination with glucose. Galactose utilization was enhanced when it was used with glucose, but



did not match the growth in the fructose-plus-glucose system. This difference was more pronounced in cultures under dark than in light. The presence of either arabinose or xylose with glucose retarded the growth.

Comparing these results with those of Samejima and Myers (40), two anomalies are seen. Glycerol was assimilated in Theriault's studies, whereas it was not utilized at all in those of Samejima and Myers. Galactose utilization was about half of glucose utilization in the latter, compared to one-fourth in the former. Two explanations are possible for these anomalies. Samejima and Myers used the Emerson strain of Chlorella pyrenoidosa, whereas Theriault used Chlorella pyrenoidosa 7-11-05 (Sorokin). The strain 7-11-05 adapted to galactose after six transfers. The efficiency for utilization of galactose in this adapted strain improved to fifty-eight per cent of the glucose utilization, while that of the wild strain was about ten per cent. Theriault's further study with single carbon sources was accomplished under illumination. Since there was neither a CO<sub>2</sub> supply nor bacterial contamination, the growth was organotrophic. The presence of light might have had some effect.

Lewin (42) selected a facultatively heterotrophic species, Chlamydomonas dysosmos, and obtained a mutant (D.2075) induced by ultraviolet light, which behaved like an obligate photoautotroph. The growth of the wild type in the dark was good on acetate, slight on lactate or pyruvate,

and absent on other sugars tested, including glucose. The mutant, D.2075, did not grow on acetate, lactate, or pyruvate. Lewin concluded that the assimilatory system for acetate was present in the wild type, and absent in the mutant.

Inability of all of the sixteen known species of Chlorococcum to utilize either glucose or acetate has been reported (43). This quality of obligate photoautotrophy can be used to distinguish this genus from others. Nevertheless, in some other genera some species are heterotrophs and other species are autotrophs. Two strains of Chlorella vulgaris have been shown not to grow in the dark on glucose (one per cent solution) (44) (45). The growth did not stop immediately when cells were transferred from light to dark, but slowly decreased up to five days, after which it was completely stopped. To study the pigments produced by Chlorella vulgaris in darkness, Myers (46) grew it on 0.5 per cent dextrose and 0.2 per cent peptone. He found that the pigments produced by this strain of Chlorella vulgaris in darkness were the same as that produced in the light. Possibly peptone was used as substrate, or pigment production might have taken place without growth; no data were presented with respect to cell numbers, but it would appear from the results which were presented that production of the pigment was not dependent upon light energy.

In a study on algal growth in sewage oxidation ponds, Allen (32) made the following statement:

"It at first appeared possible that the dominance of Chlorella in the ponds could be due partly to its ability to grow during the hours of darkness. However, numerous experiments have shown that neither Chlorella nor other common pond algae (Scenedesmus, Chlamydomonas, Ankistrodesmus) can grow oxidatively on sewage in the dark, whether alone or in the presence of bacteria, so that it must be concluded that neither sewage nor its bacterial oxidation products contain materials utilizable by Chlorella in the dark in the pH range encountered in oxidation ponds. It was also found that growth of Chlorella on sterilized sewage in the light did not result in any decrease in oxidizable organic matter."

While convincing data were presented for the second finding, no data were shown for the first conclusion based on numerous experiments.

As if to verify the above statements, Pipes and Gotaas (47) made an attempt to determine the organic compound(s), if any, in sewage which can be oxidized directly by Chlorella. It is of interest to note that these workers used a strain of Chlorella pyrenoidosa isolated by Allen, though her work was not referenced. They conducted pure culture studies in continuous flow units under saturating light intensity (1000 ft-c) using sterile filtered sewage supplemented with 0.0 per cent, 0.03 per cent, and 0.1 per cent CO<sub>2</sub> air mixture. After the cultures had attained equilibrium, the dissolved volatile solids and BOD in the influent and effluent supernatant were determined. Any change in the concentration of these parameters was attributed to algal metabolism. They concluded that cultures of Chlorella pyrenoidosa having detention periods of

less than three days did utilize some of the organic matter present in sewage, and that older cultures (detention times of more than three days) excreted organic matter into the medium. The design of the experiment did not provide an ideal test, since provision of  $\text{CO}_2$  and light energy led to photosynthetic growth which introduced an unnecessary complicating factor. Hence, the experiments with 0.03 per cent and 0.1 per cent  $\text{CO}_2$  air mixture do not merit consideration with respect to assessing the utilization of organic substances by the algae. In the figures shown some points were not plotted at all, and some were incorrectly plotted. Considering the series with no addition of  $\text{CO}_2$ , the BOD and dissolved volatile solids values were less than the sewage value only for the cultures of one-day detention periods. For all other cultures, these values were equal to or more than the feed sewage. Furthermore, in the cultures having detention periods of more than two days, the BOD, dissolved volatile solids, and packed cell volumes were all increasing. The authors did not explain how this could happen. From a scrutiny of the basic data it seems that the reduction of alkalinity in these systems was the explanatory factor for the above phenomenon. The increase in pH gives additional support for this surmise. Because of the above criticism, the authors' conclusion about the utilization of organic matter would appear to be based upon inadequate data, and therefore their conclusions are of questionable value. However, their conclusion that the increase in the

supernatant BOD was due to secretion of organic substances by the algal culture of higher detention periods is supported by their data.

Pipes (48), the senior author of the paper discussed above, possibly was not certain of the findings. He later designed an investigation "to provide information which might indicate if stabilization pond algae do actually assimilate organic matter from waste waters," and other related conditions. He used the same organism, Chlorella pyrenoidosa, cultivated in an organic medium enriched with 0.03 per cent and 1.0 per cent CO<sub>2</sub> air mixtures under light. Growth rates in media with and without many organic substances were determined. The difference in the growth rates was attributed to the organic matter added. The increase in growth rates was more pronounced in systems with 0.03 per cent CO<sub>2</sub> than in the 1.0 per cent system. In the latter, the increase in growth rates was observed with organic nitrogen sources such as dilute urea. Pipes fractionated sewage into six parts, as shown in Table I, and tested them separately. The water-soluble fraction increased the growth rate. Here, also, the increment of growth was more apparent in the system aerated at the low CO<sub>2</sub> tension.

Solook (49) studied the responses of a mixed algal culture (predominantly Chlorella) to glucose. He developed the algal culture photo-autotrophically, added glucose, placed the culture in the dark, and tested for glucose

removal. He added no bacteria to the system, but did not use aseptic techniques. He stated that "the only bacteria present were those occurring as natural contaminants in the system." He noted an initial lag period in substrate removal. He attributed this lag to "the fact that because the microbial population had been developed photo-autotrophically, the needed enzymes for heterotrophic metabolism had to be synthesized before substrate utilization." Another explanation, as given below, seems possible. The lag represented the time taken by the very few contaminating bacteria in the culture to multiply and attain a number sufficient to exert an observable utilization of the substrate. This surmise can be supported by Solook's statement that "in all systems there was a good correlation between the biological solids concentration and the viable bacterial counts." His observation indicated that bacterial growth occurred in all systems; therefore, the substrate removal which was observed could have been due entirely to bacterial growth from an initially very small population (which would account for the apparent lag in substrate removal).

In reviewing the literature on heterotrophic metabolism of algae, attention has thus far been focused on utilization of organic substrates without special attention to algal respiration. However, algal respiration, and in particular endogenous respiration, and the effects of various factors (e.g., intensity of light, organic

substrate, etc.) on them are important considerations which require further discussion in regard to depletion of the dissolved oxygen resource.

Gibbs (50) assembled and assessed the literature on algal respiration. Discussing the effects of added substrates on endogenous respiration, he pointed out that studies employing manometric techniques yielded evidence that the endogenous respiration is suppressed, whereas studies using isotopically labeled cells indicated that endogenous respiration is either unaffected or is somewhat stimulated. He concluded that "the effect of an external oxidizable substrate on the endogenous respiration is thus still unresolved. It is not unlikely that the situation may differ from one species to another, or within one species, according to the physiological state of the cells."

Solook (49) added 50, 100, and 125 mg/l of glucose to a photosynthetically grown Chlorella pyrenoidosa culture, and followed oxygen uptake in a Warburg respirometer. He calculated values of  $RO_2$ , rate of oxygen uptake in milligrams of oxygen per hour per gram of solids, and found that they were approximately equal for all three loadings. For the limited purpose of comparing the  $O_2$  uptake for the different substrate levels, the above unit of expression is quite sufficient. However, the unit implies that the oxygen uptake curve more or less followed a straight line, i.e., zero order kinetics, during the whole period for which the rate was calculated. The oxygen uptake curves were not

shown for these particular experiments; however, for other similar experiments the  $O_2$  uptake curves were shown. The uptake rate was higher at first, and then (probably after exhaustion of the substrate) reduced considerably. The values of cumulative oxygen uptake (mg/l) for a system with 150 mg/l glucose as taken from his Figure 10 were 37, 64, 83, 90, and 97 at 20, 40, 60, 80, and 100 hours. The increments of oxygen uptake were 37, 27, 19, 7, and 7 mg/l for each twenty hours. It can be seen that the uptake rate reduced considerably after sixty hours. This reduction in the  $O_2$  uptake rate  $RO_2$  (mg  $O_2$ /hr/mg solids) would be more pronounced when the increase of the solids in the system was taken into consideration. The results would have been more meaningful had the  $RO_2$  been calculated for the two portions of the curve, namely, for the periods during the active utilization of the substrate and after the apparent exhaustion of the substrate.

Allen, et al. (51) and Fitzgerald (52), using Chorella pyrenoidosa (Wis 2005) concluded that the respiration value depended on cell age and the nature of the culturing medium. Kuttyurin, et al. (53) working with Chlorella and Elodea found that the respiration rate in the dark was directly proportional to the oxygen concentration. However, Myers (54), experimenting with Chlorella pyrenoidosa (Emerson's strain), found that the time for utilization of glucose was unaffected by the simultaneous occurrence of photosynthesis or by illumination in the



absence of carbon dioxide. The data of Ludwig, et al. (30) showed that the respiration (unit  $O_2$  uptake) of Euglena gracilis decreased as the retention period in a continuous flow unit was increased to fourteen days. At detention periods greater than fourteen days, algal respiration gradually increased, and finally, at a retention period of twenty-one days, it was equal to that of the three-day retention period.

#### Excretory Products

Excretion of organic substances into the medium by algae adds an additional load to the oxidation pond, and could adversely affect the efficiency of the system. The excretion of organic matter works in opposition to the heterotrophic properties of algae in oxidation ponds. Estimation of the quantity and the identification of the excretory products and their possible effects on the purification process is reviewed in some detail below.

Tolbert and Zill (55) made studies on Chlorella pyrenoidosa using  $C^{14}$  as a tracer. They found that glycolic acid, amounting to three to ten per cent of the total  $C^{14}O_2$  fixed, was excreted into the medium during short term photosynthesis. The concentration did not increase beyond 3-9 mg/l. This glycolic acid was re-absorbed by the cells, when the bicarbonate fixation stopped. They postulated that a bicarbonate-glycolate anion equilibrium existed across the cell membrane. Since this was a pure culture study, there was no possibility for

the glycolate in the medium to be utilized by any other microbes. However, if the system had contained glycolate-consuming organisms, it can be seen that the equilibrium would have been disturbed, resulting (possibly) in further excretion of glycolic acid, which would, in turn, increase the total organic load on the system.

Allen (56) investigated the excretory behavior of six species of Chlamydomonas, another genus commonly found in oxidation ponds. The results showed that liberation of organic substances was proportional to the dry weight of the algae in all five cases tested. The quantity was greater at higher light intensity and when  $\text{NO}_3$  was the nitrogen source. She identified glycolic acid and pyruvic acid by paper chromatography; the presence of polysaccharide was also established.

Lewin (57) isolated eighteen species of Chlamydomonas from soils and mud at the borders of water bodies in seven different countries. Only one of them had been tested previously by Allen. He concentrated on the identification of extracellular polysaccharides produced by them. Soluble organic matter excreted by one of the three non-mucilaginous species was as high as eight per cent of the total organic matter (cells + polysaccharide). The maximum quantity, twenty-six per cent (excluding capsular material) was excreted by Chlamydomonas mexicana, a mucilaginous species. Arabinose and galactose were found in the polysaccharides excreted by seventeen of the

eighteen species examined. Fucose, xylose, and glucose were components of the polysaccharide in some systems.

Conducting investigations with a thermophilic strain of Chlorella pyrenoidosa and a mesophilic culture of Chlorella vulgaris, Maksimova, et al. (58) studied the phenomenon of secretion of organic substances. They also found that the higher the yield of algae, the greater is the quantity of material excreted into the medium. The two species tested behaved in the same manner.

Comparing the works of Maksimova, et al. (58), Lewin (57), and Allen (56) there were two significant differences in their findings. One difference pertained to variation in the percent of soluble organic material elaborated per unit of cell mass in a given time. Maksimova, et al. found that this value was approximately thirty per cent during the first two days, and decreased to five to ten per cent thereafter. The data of Allen and Lewin are not sufficient for an appraisal of the above change with respect to time. The indicated data did not show any significant variations. Whether this difference can be attributed to the difference in the genera Chlorella and Chlamydomonas or to any other reason is not clear.

Another difference was related to the light intensity. Maksimova, et al. concluded that light intensity had no appreciable effect on the quantity of excretory products in contrast to Allen's finding of increased quantity under higher light intensities. Both conclusions were based upon

observations at only two light intensities. Maksimova, et al. used 5000 lux (500 ft-c) and 10,000 lux (1000 ft-c), whereas Allen used 25 ft-c and 750 ft-c. There was no appreciable change in the first case, and the change in the second was six-fold. From this limited data it seems that the process of excretion, too, may have a limiting light intensity at or below 500 ft-c. If one takes as the limiting light intensity a value at or near 400 ft-c, the effects observed below 400 ft-c will be dependent on light intensity, whereas the effects observed above 400 ft-c will be independent of light intensity.

Maksimova and Pimenova (59) extended the above study, and determined qualitatively the composition of organic matter excreted by the algae. They did not find any nitrogen-containing compound in the filtrate. The constituents of the water-soluble polysaccharides were galactose, mannose, arabinose, xylose, ribose, fucose, and rhamnose. In addition, traces of glucose, fructose, and sucrose were detected.

Merz, et al. (60) assayed the extracellular products of twenty-two species of Chlamydomonas, Chlorella, and Scenedesmus. Of these, six were isolated from oxidation ponds. The  $\text{CO}_2$  in the compressed air (0.03 per cent) was the carbon source. The COD of the supernatant medium was determined. Separation and qualitative assessment of products were made by paper chromatography. The COD of the soluble extracellular organics of ten systems was greater

than 100 mg/l, and in one system the COD was approximately 500 mg/l after twenty-four days. The average daily increases in COD concentration for Chlorella pyrenoidosa, Chlorella vulgaris, and Chlorella miniata were 5.43, 4.19, and 14.86 mg/l, respectively. These values for two species of Chlamydomonas were above 5 mg/l. The identified products included galactose, fucose, ribose, glucose, malic acid, oxalic acid, and glycolic acid.

Surprisingly, Merz, et al. concluded that "the effect of excretion of extracellular, soluble organic matter by algae on the efficiency of an oxidation pond is not likely to be significant." Many works, including their own, had shown that excretory products are of considerable quantity and of wide variety. It is quite reasonable to expect that these will have a significant effect on the efficiency of the pond; however, they did not conduct any experiments to arrive at their conclusion. Apparently they felt that 100 mg/l additional COD was an insignificant amount.

The growth of several microorganisms in cultures of Chlorella pyrenoidosa to which no organic substrate was added was studied by Vela and Guerra (61). The algal population and the "contaminating" (added) bacterial numbers were counted. The proliferation of bacteria was observed only when the algae were actively dividing. The organic compounds in the medium were fractionated, and they were tested in the Warburg respirometer for capability of supporting bacterial growth. The results showed that these

organics supported growth of bacteria, the rate being different for different species of bacteria. Ninhydrin-reactive materials and organic acids were detected by chromatographic analyses. The accompanying mass culture study indicated various types of contaminant bacteria, each type predominating at different ages of the algal culture.

### Inhibition

Retardation and inhibition of algal and bacterial metabolism may occur due to compounds present in oxidation ponds, resulting in decreased efficiency. Such compounds may be either brought into the ponds in the influent itself, or produced by microbial metabolism. Since, in the present research, the inflow quality was the same except for small changes in the components of the tap water, discussion is centered on the second source, namely, inhibition by excretory products of the microbial population.

Pratt and co-workers studied the growth of Chlorella vulgaris under continuous lighting in a nutrient medium, through which a five per cent CO<sub>2</sub> air mixture was bubbled. Their data indicated that: (1) the cells excreted a substance which retarded their own growth (growth was measured by increase in cell numbers) (62), (2) the growth-retarding substance, designated as Chlorellin, exhibited antibacterial properties against five tested bacteria, as seen by the zones of inhibition in the standard cup assay (63), and (3) the concentration of Chlorellin was at a minimum during rapid growth (64).

Shilo (65) reported that synthesis of toxic substances in Prymnesium parvum was greatest during the late log growth and stationary phases. Karlander and Krauss (66) attempted to determine the reasons for absence of growth of Chlorella vulgaris in the dark in the presence of glucose. Noting a decrease in pH of approximately two units, they suspected the excretion of organic acids. By gas chromatographic analysis of the supernatant, they identified acetic acid and formic acid as two of the components excreted in the dark. However, further experiments indicated that secretion of acid was one of the results of inhibition, and not the cause of it.

Pipes (48) measured the growth rates of Chlorella pyrenoidosa (cultured photosynthetically) in media enriched with various organic substances, including formic and acetic acids at three concentrations each. The specific logarithmic growth rates ( $k_g$ , day<sup>-1</sup>) are given below:

<u>Compound</u>	<u>Concentration</u>			
	0	10 <sup>-3</sup> M	10 <sup>-4</sup> M	10 <sup>-5</sup> M
glucose	1.400	1.490	1.460	1.465
formic acid	1.390	1.015	1.344	1.385
acetic acid	1.410	1.180	1.380	1.390

It can be seen that formic and acetic acids did have a retarding effect at the higher concentrations employed.

Leone (67) conducted an experiment for seventy-two days with Chlorella pyrenoidosa strain 7-11-05 in which the medium was recycled. He concluded that even though some

amount of autotoxic substances might have been produced, no buildup of them was noticed.

Oswald, et al. (31) found the BOD values of the supernatant from a Chlorella culture were higher than those of the supernatant from an Euglena culture. They attributed this result to the lower bacterial population in the former system. Their data are given below:

<u>System</u>	<u>Bacterial Colonies per ml</u>
1. Sewage + bacteria	$1 \times 10^8$
2. Sewage + bacteria + <u>Euglena</u>	$1 \times 10^7$
3. Sewage + bacteria + <u>Chlorella</u>	$1 \times 10^6$

As an explanation for the lower bacterial count in system 3, they reasoned that "some factor inhibits bacterial action upon the sewage substrate while the Chlorella cells are present." While the given explanation is quite possible, another reason also seems plausible. When there are two types of organisms capable of using the substrate in the system, there will be competition between them for the substrate utilization. The ultimate population reached by either type of organism in the combined system will be lower than that of the population attained by each organism when it is present alone. Substantiation for this line of reasoning is obtained by comparing systems 1 and 2, or 1 and 3. It is normal to expect that faster-growing organisms will attain higher numbers in the combined system; however, the numbers reached by the faster-growing organisms depend upon the relative growth rates of the two types



of organisms. Euglena has a lower growth rate than that of Chlorella. This fact was recorded by the authors themselves in another paper (30). As a hypothetical example, if Euglena had utilized twenty per cent of the substrate during the experimental period, Chlorella would have used forty per cent of the substrate, leaving eighty per cent and sixty per cent for the bacteria in systems 2 and 3, respectively. This would lead to a lower bacterial population in systems 3 than in system 2.

Discussing the reason for occasional low BOD removals in high rate ponds, Oswald, et al. (68) suspected the existence of higher pH values as the major reason. This explanation may be plausible and it could have been confirmed had the authors given data correlating pH values and BOD removal. Fitzgerald, et al. (69) studied the toxicity of approximately three hundred organic compounds on Microcystis aeruginosa, a bloom-producing species. Gloyna and Thirumurthi (70) tested the toxic effects of about sixty organic chemicals on Chlorella pyrenoidosa. The general pattern indicated by their results was that the straight chain compounds were more toxic than corresponding branched chain compounds. Among the straight chain fatty acids, those with odd numbers of carbon atoms possessed greater toxicity than those of even number. Furthermore, they found that the toxic effect of some chemicals can be nullified by certain inactivating chemicals.

Kott, et al. (71) studied the inhibitory effect of

chlorine and bromine on Chlorella pyrenoidosa. At a concentration of 0.4 ppm, both exhibited algicidal properties; the effect of bromine was greater than that of chlorine. This indicates that the presence of these residual halogens in the influent to an oxidation pond will affect the photosynthetic oxygenation process.

#### Efficiency of Oxidation Ponds

All of the factors and processes hitherto discussed in detail, and others, such as the type of waste, temperature, and bacterial metabolism, can be expected to affect the overall efficiency of oxidation ponds. The performance of the pond can be evaluated by employing various parameters, namely, oxygen demand satisfied, solids reduced, nutrients removed, etc. The selection of a particular parameter depends upon the purpose for which the pond is intended. Most oxidation pond installations are intended as secondary treatment devices. In those, reduction of oxygen demand is the usual, though not the exclusive, yardstick of measurement for efficiency.

Organic loading is reckoned by two parameters: BOD and COD. While it is not the intention of the author to discuss the advantages and limitations of these two parameters, some mention of the relation between them, with particular reference to oxidation ponds, is warranted. BOD is monitored extensively in the field, since these data are usually required by the regulatory agencies, while COD is used in an increasing number of cases in laboratory studies.

To compare studies using these two different parameters and to extrapolate the findings of numerous laboratory studies to the field conditions, correlation between BOD and COD would be quite useful.

Ballinger and Lishka (72) evaluated the BOD and COD of a sample containing glucose and glutamic acid (1.6 grams each in one liter) with a theoretical oxygen demand of 308 mg/l. The 5-day BOD value was 186 mg/l, and the COD was 281 mg/l; i.e., the  $BOD_5$  was sixty-six per cent of the COD: however, this ratio is not a constant one. It is not only different for different wastes, but varies with the relative proportions of the constituents at various times within a particular waste. Loehr (73) determined the oxygen demand (BOD and COD) of the effluent from a lagoon treating animal feedlot wastes:

$BOD_5$ (mg/l)	1340	1420	1930
COD (mg/l)	4700	5500	7400
$BOD_5/COD$ (%)	28.5	25.8	26.1

McKinney (74) reported on the performance of an aerated lagoon treating predominantly domestic wastes. The median values of the parameters were:

	<u>Influent mg/l</u>	<u>Effluent mg/l</u>	<u>Reduction %</u>
$BOD_5$	125	26	80
COD	360	165	54

The difference in the expression of performance was due to the difference in the BOD/COD ratio of the influent and effluent, which were 34.7 per cent and 15.7 per cent,

respectively. In an oxidation pond used in Kansas for the treatment of oil refinery wastes (75) the oxygen demand removed during summer and winter were eighty-three per cent and fifty-two per cent on the basis of BOD, and sixty per cent and thirty-eight per cent on the basis of COD. The percentage removal of oxygen demand in an oxidation pond receiving influent from trickling filters is given below (76); the determinations were made on seven days in a period of ten months:

As BOD	50	20	58	50	59	44	68
As COD	-2	2	0	22	7	7	39

The absolute COD values for the first reading giving negative reduction were 3232 and 3295 for influent and effluent, respectively, and showed an increase of 63 mg/l in the pond.

Results of a pilot plant study employing pasteurized partially skimmed milk as substrate have been reported (9). The detention time was ten days for these (semi-continuous) fill-and-draw type units, which were fed intermittently on alternate days. The 5-day BOD of the pond water immediately after a feeding ( $BOD_i$ ), and just before the next feeding after two days ( $BOD_e$ ), and the corresponding percent removals between feedings ( $R_p$ ) were as follows:

$BOD_i$	49	84	56	56	76	82	59	60	74	95	70
$BOD_e$	56	24	37	58	60	48	42	58	76	49	66
RB %	-14.3	71.4	34.0	-3.6	21.0	41.5	28.8	3.3	-2.7	48.5	5.7

The average BOD reduction in the test period of twenty-two days was only 21.2 per cent. The reduction, had it been

expressed on a COD basis, would have been still less (possibly negative).

Oswald, et al. (31) presented data on  $BOD_5$  of the influent sewage, the unfiltered effluent, and of the culture supernatant, at various retention periods. The whole effluent  $BOD_5$  increased with time of retention; for 1-day cultures it was about ninety per cent of the influent sewage, equal to the influent  $BOD_5$  for 2-day cultures and almost double in 7-day cultures. However, the BOD of the clear supernatant had a value of about forty-five per cent of the influent.

Pipes (77) studied BOD removal in laboratory stabilization ponds operated at various levels of pH, using synthetic media. The results showed two opposing trends of BOD reduction as a function of detention periods. When the pH was above or equal to 8.0, the BOD reduction increased with increased detention time, but when the pH was below or equal to 7.5, the effluent BOD increased with additional retention periods. It should be noted that in a system of given total alkalinity, an appreciable quantity of free  $CO_2$  is present when the pH is approximately 7.5 or lower.

Hermann and Gloyna (78) investigated two identical laboratory model oxidation ponds exposed to outside weather conditions in Austin, Texas. The daily batch feeding in one of them was double that of the other, resulting in a detention time of ten days in the former and twenty days in the latter. The BOD removals were ninety-eight per cent

and ninety-three per cent in low and high detention ponds, respectively. The authors noted this, and stated that "this phenomenon may be attributed to a considerable increase in algal population." Though not complete, their reasoning seems to be correct. The increased algal population in ponds with higher detention periods would have assimilated more  $\text{CO}_2$  from the atmosphere, and presumably would have secreted more organic substances. Further argument against the use of high detention times may be provided by analysis of the pond system treating oil refinery wastes referred to earlier (75). This pond system consisted of several ponds in a series, and it was possible to calculate BOD removal at various detention times. The maximum reduction in BOD occurred at approximately 20-day detention time, after which the effluent BOD increased. The authors indicated that "peak algal populations occurred in this region, and it is probable that the apparent BOD reduction rate was diminished after this time by the addition of organic compounds from algal decomposition." Some decomposition along with the excretion of oxidizable material could have caused the BOD increases which were observed to occur.

Wu (79) studied the effect of various organic loadings in laboratory oxidation ponds operated with detention periods of ten and twenty days. He concluded that the maximum allowable loading was near 62 lb/COD/acre/day for the maintenance of aerobic conditions and for reasonably good COD

removal efficiency. In his investigation he conducted two experiments with equivalent loading at the different detention periods, i.e., one with 300 mg/l at ten days detention, and the other with 600 mg/l at 20-day detention time. The percentage COD removal was greater in the 10-day detention pond (300 mg/l) than in the 20-day detention pond (600 mg/l). Furthermore, the "steady-state" dissolved oxygen levels were 4 mg/l in the pond with the shorter detention period (10 days) and 1 mg/l in the pond with the longer detention time (20 days).

## CHAPTER III

### THEORETICAL CONSIDERATIONS AND EXPERIMENTAL APPROACH

From the review of the literature it is apparent that for many cases the  $\text{CO}_2$  concentration was the growth-limiting factor for the algae (30) (31) (32) (33). In many instances the  $\text{CO}_2$  produced by bacterial metabolism might be expected to increase the  $\text{CO}_2$  concentration to values in excess of those needed for algal growth. However, this active bacterial oxidation occurs, in general, in the influent end of the pond. The excess  $\text{CO}_2$  produced can escape to the atmosphere, and hence all bacterial  $\text{CO}_2$  might not be fully available to the algae. Furthermore, it was seen that the minimum concentration of organic substances occurs in the middle of the pond, and the BOD often increases as the waste water progresses through the pond (75) (78). It was pointed out that the probable reason for this phenomenon is the continued growth of the algae and subsequent excretion of organic substances by them.

The oxygen balance could be improved by the increased algal utilization of  $\text{CO}_2$  produced by bacteria near the influent end of the pond and the increase of the BOD in the effluent end of the pond could be reduced by bringing these two phases together, i.e., possibly by more intimate



mixing. Without more intimate mixing, there would always be the tendency for bacteria to predominate where the organic substrate concentration is higher, namely, at the influent end, and for the algae to be in abundance in areas of less turbidity, (hence more light) at the effluent end. Any engineering expedient to counteract this natural separation and to keep these two phases together by supplying mixing energy results in additional expense. Also, mixing, if too turbulent, would strip  $\text{CO}_2$  and  $\text{O}_2$ , thus militating against its possible advantages. It is possible that the desired advantages could be achieved by an alternate means. For example, if a pond were closed to the atmosphere (but not shielded from light), the super-saturation of  $\text{CO}_2$  and  $\text{O}_2$  could not be relieved by escape to the atmosphere. This concept for oxidation pond operation might be practically engineered by employing a transparent covering material such as polyethylene. It seems possible that the advantage of the "closed oxidation pond" with respect to oxygen transfer might even outweigh the advantages with respect to  $\text{CO}_2$  tension. The rates of oxygenation and deoxygenation depend on the difference of  $\text{O}_2$  concentration in the pond and in the atmospheric air. In an aerobic oxidation pond the DO in the early morning hours would be near zero. The maximum oxygen gain to the pond is due to a driving force equal to the solubility of  $\text{O}_2$  in the medium, i.e., approximately 8 mg/l. It is quite common to encounter DO concentrations of 20 mg/l and above during the afternoon

hours. Some of this excess (around 12 mg/l) dissolved oxygen is lost, due to stripping, relieving the supersaturation. On the whole, the open pond may therefore lose oxygen to the atmosphere, a situation which could be prevented in a closed pond. Furthermore, the curtailment of evaporation loss in the closed pond would be an additional advantage in areas where water is scarce and the stream flow consists largely of the pond effluent.

Thus far in this development of some of the theoretical concepts pertinent to oxidation pond operation, it has been assumed that algal aeration is more critical than  $O_2$  transfers across the liquid-atmospheric interface. Also, it has been assumed that transfer of  $O_2$  to the atmosphere takes place at a rate approximately equal to transfer into the liquid. The same line of reasoning has been assumed for  $CO_2$ , although the problem here is not so critical because of the carbonate balance reactions which, depending upon the pH, may entrap some  $CO_2$ . In addition, the driving force for transfer of  $CO_2$  into the liquid is very small, and one need not consider  $CO_2$  from the atmosphere as a major contributor to the  $CO_2$  pool.

An experimental verification of the hypothesis that an open pond may lose excessive amounts of oxygen to the atmosphere leads to the need for determination of reaeration rates for the experimental ponds. Recently, Isaacs and Gaudy (80) have shown the necessity of determining the true saturation values for obtaining correct  $K_2$  rates. Hence,

in addition to the measurement of the apparent saturation values, a check by the method described by Isaacs and Gaudy (81) would provide a useful tool for the present research. For the determination of the saturation values in the reaeration experiments, the equations of Isaacs and Gaudy given below could be used directly:

$$\alpha = \frac{(D_1)(D_2) - (D_3)^2}{D_1 + D_2 - 2D_3} \quad (3)$$

$$C_s = C'_s - \alpha \quad (4)$$

where

$C_s$  = the true saturation value

$C'_s$  = assumed saturation value

$\alpha$  = error in the assumption, or the correction factor

$D_1, D_2, D_3$  = apparent deficits at times  $t_1, t_2$ , and  $t_3$

$$\text{when } t_3 = \frac{t_1 + t_2}{2}$$

However, for the determination of the saturation values in deaeration experiments, only Equation (3) can be used directly, and Equation (4) must be modified as follows:

$$C_s = C'_s + \alpha \quad (5)$$

While at first sight this may seem contradictory, the following analysis will show the equivalence of both equations. Consider two hypothetical systems having the same  $K_2$  rates. One system is reaerating, and its true saturation value is 8 mg/l. The other one is deaerating, and its true saturation value is 10 mg/l. Figure 2 shows the reaeration and

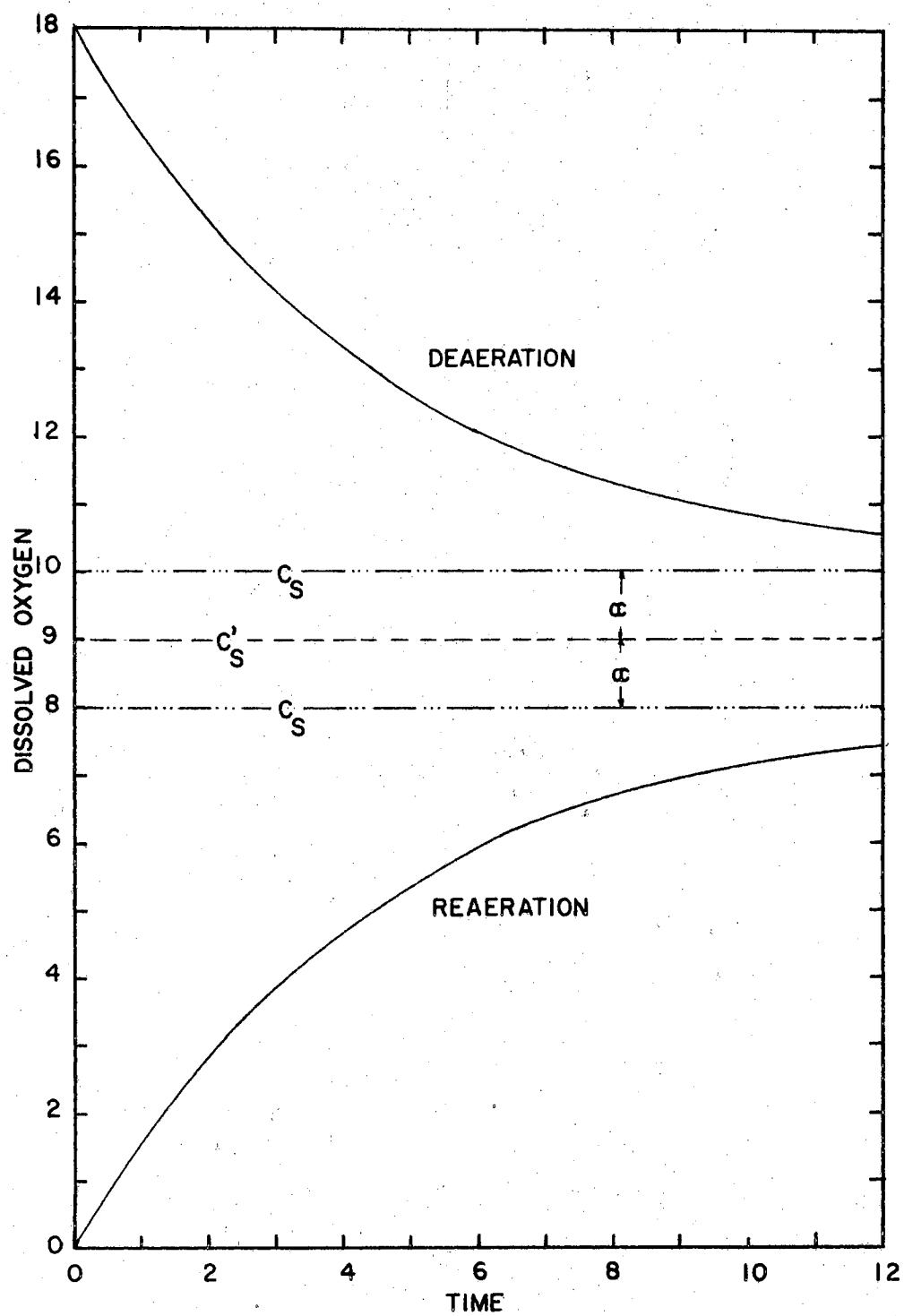


Figure 2 - Reaeration and deaeration curves of two hypothetical systems of the same  $K_2$  rates.

deaeration curves for the systems. Let the assumed saturation value for both the systems be 9 mg/l. The value of  $\alpha$  which could be calculated using Equation (3) is 1. To get the saturation values, this must be deducted from the assumed value of 9 mg/l in the reaerating system, whereas it must be added for the deaerating system.

The correction factor  $\alpha$  is the distance of the asymptotic line from the line of assumed saturation, and represents the difference in DO levels between the true and assumed values. The value of  $\alpha$  will be positive when the two lines do not cross, and negative when they cross. The reference point is the line of assumed saturation for both the deaerating and reaerating systems. However, the true saturation levels of the two cases lie on opposite sides of the reference line. Hence, the sign change in Equations (4) and (5). This change in sign can also be explained by taking into account the direction of measurement. The Y axis, on which DO concentrations are plotted, is positive upward. The deficits in the reaeration curve are measured downward from the saturation line, whereas the excesses in the deaeration curve are measured upward from the saturation line. In one case (deaeration), the differences in concentration are measured along the direction of the axis, and in the other (aeration) they are measured against the direction of the axis. Hence the sign of  $\alpha$  is different in the two cases.

In oxidation ponds a steady state condition with

respect to oxygen concentration (in the strict sense of the word) cannot be achieved, since the light intensity (an energy yielding "substrate" for phototrophic organisms) is subject to constant change. Nevertheless, since the light and dark periods alternate with some degree of regularity, a cyclic pattern, increasing during the day and decreasing during the night, can be expected. In laboratory experiments where the factors such as the influent, detention time, and lighting can be controlled, this regularity can be made more rigorous.

In such controlled studies it might be expected that the system would be approaching a "balanced condition" wherein the DO increase during the light period might be more or less equal to the DO decrease during the dark period. The fluctuation in DO might be represented as shown below:

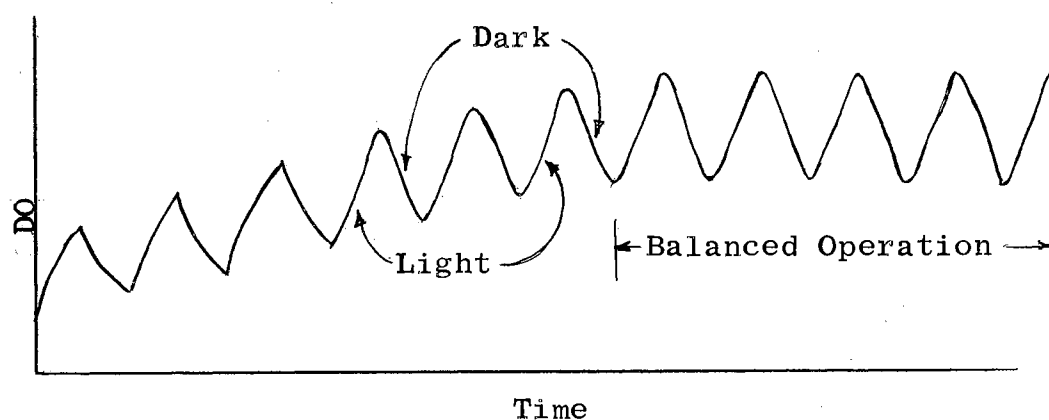


Figure 3 - Dissolved oxygen in a pond under balanced operation.

This figure is intended to show a general pattern, and does not necessarily represent the kinetic mode of change during the light and dark periods.

#### Experimental Approach

The dissolved oxygen concentration present in oxidation ponds is a result of two groups of opposing forces, one adding to and the other deleting from the oxygen resource. The significant processes tending to increase the DO are the physical transfer of oxygen from the atmosphere into the pond where the DO in the pond is below saturation and the photosynthetic oxygenation by algae during the light period. In the other group, which reduces the DO concentration, the most important processes are utilization of oxygen by the organotrophic bacteria, the possible organotrophic metabolism of the algae, and the loss of oxygen by stripping during the period of supersaturation.

The experimental approach taken in the present research was one which attempted, insofar as possible, to assess the course of each process independently. In order to gain deeper insight into a few of the constituent processes, additional investigations were made. To relate the findings to previous studies on oxidation ponds in the bio-environmental engineering laboratories of Oklahoma State University by Wu (79), substrate loadings employed in these studies were in the general range of those employed therein. During the course of the present research effort, some experiments were conducted in laboratory oxidation ponds of

geometric configuration similar to that used by Wu in his study of organic loadings.



## CHAPTER IV

### MATERIALS AND METHODS

#### A. Development and Description of the Equipment

The experimental equipment used in this investigation consisted of the Warburg apparatus, BOD bottles, and laboratory oxidation ponds. The description of each and the phase of study for which they were used are given below.

Pond A (Figure 4A) was an aquarium tank of the following dimensions: 48.6 x 28.5 x 27.2 centimeters with a surface area of 1385 square centimeters. The sheet glass sidings were mounted in aluminum frames, and the top was open. The tank was used for preliminary studies; rubber tube syphons were used for sampling.

Pond B (Figure 4B) was devised as an improvement on the above pond. Entry or exist ports were fitted at three levels on the narrow sides; the tank was fitted with an airtight cover. A valve and a small opening in the cover, which could be closed to the air, provided for airtightness. This tank was used in deoxygenation studies employing bacteria alone (i.e., without the presence of algae).

Pond C (Figure 4C) was a rectangular plexiglass tank of the following dimensions: 50 x 30 x 30 centimeters. The corners of the tank were joined with brass countersunk

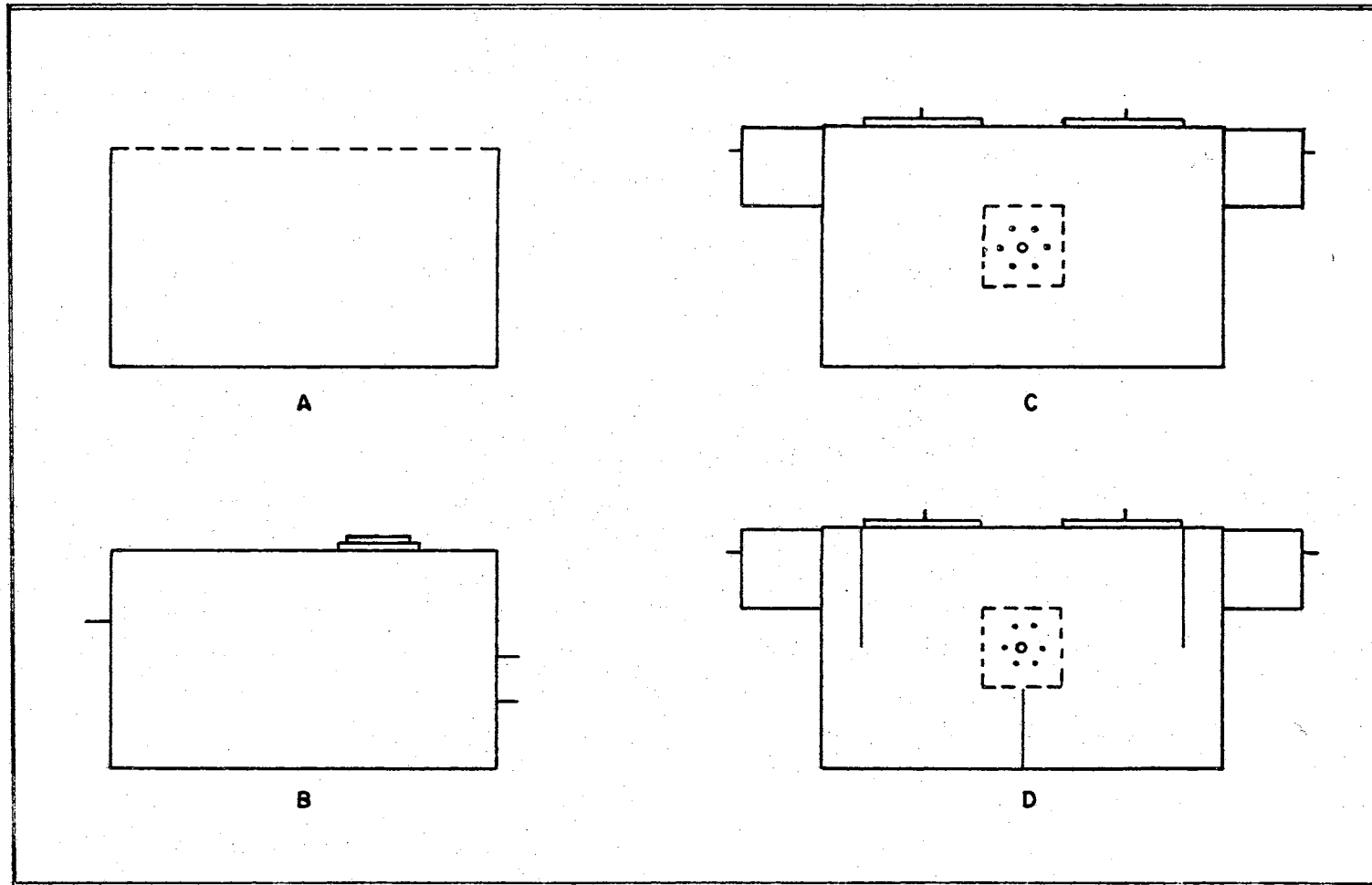


Figure 4 - Sketches of experimental oxidation ponds.

screws in addition to chemical bonding. There were three side wells 10 x 10 x 10 centimeters attached to three sides. Six holes, one centimeter diameter each, arranged in a hexagonal manner, connected these side wells to the main tank. The two side wells on the two narrow sides were fitted at the middle of the top edge, while the one on the broad side was attached at the center of the wall. All three side wells had an opening in the top, through which probes to register dissolved oxygen (electrometrically) could be inserted. In addition, the side wells were fitted with ports controlled by needle valves. Stirring magnets 2" x 3/8" were placed in all three wells. In the top of the chamber there were two 12.5" diameter openings. Lids to close these openings had valves in the center. A circular rubber ring positioned in a groove on the top surface of the tank and in the lid, and six tightening screws provided an airtight container. This tank was used in physical reaeration and continuous flow studies.

Pond D (Figure 4D and 5) was similar to C, except that it had three baffles in the main chamber. Two plexiglass baffles were attached to the top, parallel to the narrow sides, at a distance of five centimeters from each end. They extended to a depth of fifteen centimeters. Another one, ten centimeters high, was fixed to the base at the center, parallel to the other two. The provision of baffles eliminated any possibility of the formation of pockets. This tank was used in continuous flow and reaeration studies.

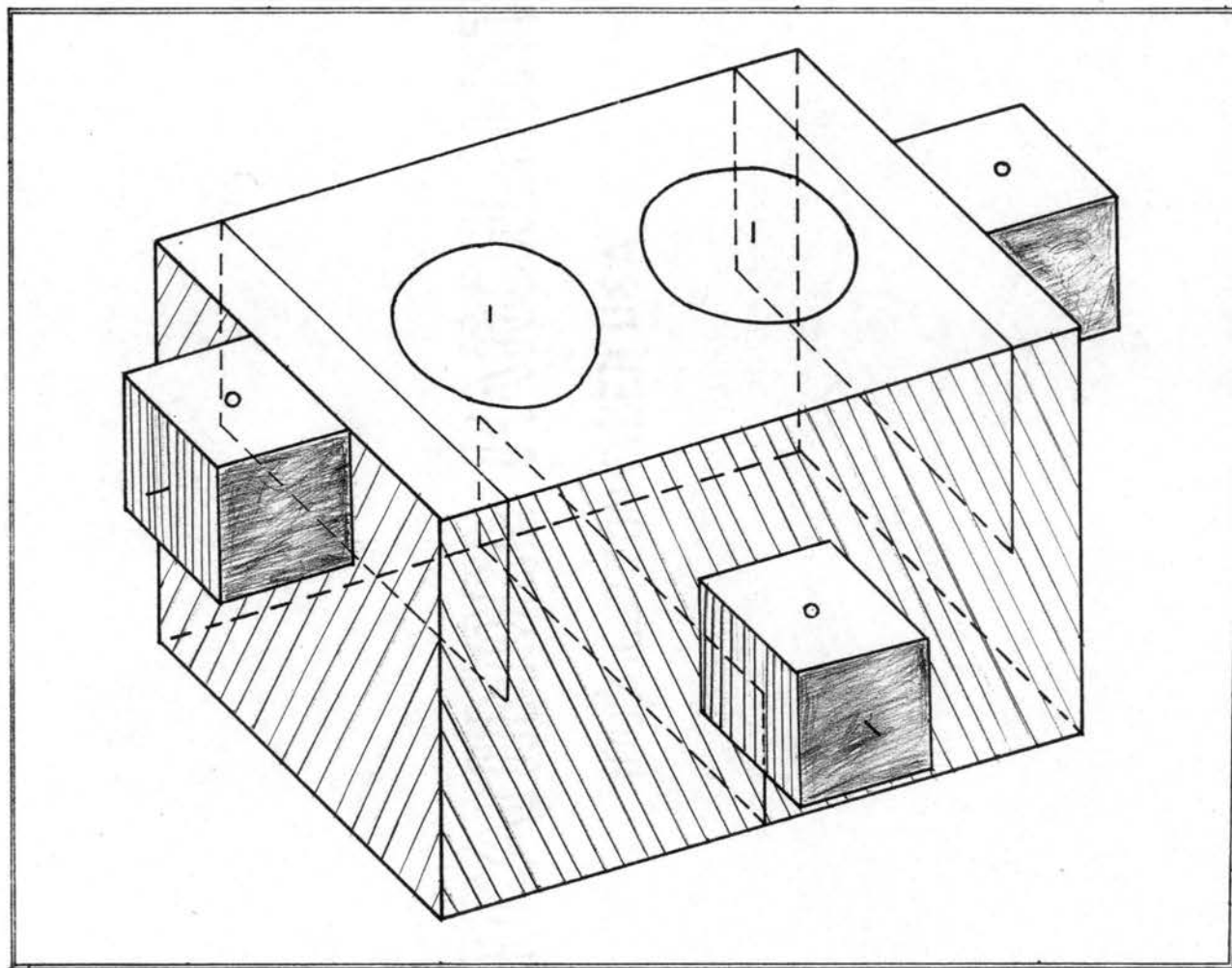


Figure 5 - Isometric view of experimental pond D.

## B. Seed

Oxidation ponds contain bacteria and algae of various species, and it would be ideal to use a heterogeneous culture of bacteria and algae. A bacterial seed was obtained from the effluent of the primary clarifier of the waste water treatment plant at Stillwater, Oklahoma, in addition, at times effluents from all of the experimental units in the bioenvironmental engineering laboratories at Oklahoma State University were mixed together and used for seeding. A heterogeneous algal population obtained from the botany department was used in the preliminary experiments. This seed caused practical (experimental) problems; the algal solids were not in uniform suspension. As a result, the reliability of the solids concentration determined by taking samples at particular locations in the pond was reduced. Furthermore, there was excellent opportunity for change in algal predominance during the long period of investigation. It was felt that heterogeneity in the bacterial population would provide ample variations. It was decided to use a pure culture algal seed which is commonly found in oxidation ponds, and which grew in even suspension.

Allen (32) reported on the specific kind and abundance of algae in sewage oxidation ponds at various localities in the State of California. Chlorella predominated under various operating conditions. Scenedesmus, Chlamydomonas, and Euglena were also found in considerable numbers.

Laboratory studies also confirmed the same trend. In Egypt a pilot plant oxidation pond study (9) was conducted for a period of five months. Chlorella, Scenedesmus, and Chroococcus were the abundant genera. Myers (82) made laboratory and field studies on fifteen sewage lagoons in Texas. He reported finding unicellular green algae and green flagellates in four ponds; colonial green algae, large spiral blue-green algae, and colonial blue-green algae in three. He did not identify the genera, but from their morphological description based upon microscopic examination, the predominant genera observed were probably Chlorella, Chlamydomonas, Scenedesmus, Spirogyra, and Gloeocarpa. Further evidence for the predominance of Chlorella in oxidation ponds is available in the literature (83) (74).

Shilo (65) reported that blue-green algae could be decomposed by a number of bacteria, while Chlorella was not affected by them. Holm-Hansen (84), giving reasons for selecting algal species for experimental material, stated: "Green algae such as Chlorella and Scenedesmus are small and unicellular, and can be readily grown in clonal culture, permitting work with larger populations which minimizes the effect of individual variation; a suspension of such algae can be pipetted accurately, and can be easily controlled in regard to temperature, light intensity, etc. Equally important is the fact that for these two genera there is an abundance of available

information on their growth and physiology."

Taking into consideration all of the above-mentioned information, it was decided to select Chlorella pyrenoidosa as the experimental alga. At the time of the present study, the agronomy department of Oklahoma State University was using Chlorella pyrenoidosa in a study to ascertain the effectiveness of some weed-controlling chemicals. They were not interested in keeping the culture pure. As such, it was possible that other species of algae might have been in their suspensions also. Seed was obtained from the agronomy department and maintained in the bioenvironmental engineering laboratory by periodic transfer into fresh medium. The seed developed in this way was used for all experiments except for those designated as pure culture studies, for which Chlorella pyrenoidosa obtained from the American Type Culture Collection was used.

#### C. Medium

In most of the research with algae, the CO<sub>2</sub> was provided by bubbling a 5 per cent CO<sub>2</sub>-95 per cent air mixture through the culture. This CO<sub>2</sub> concentration is approximately one hundred sixty times the normal concentration in air. Bicarbonate was added to the medium as an additional source of carbon, since carbonates are normally found in oxidation ponds.

In order to gain insight into the concentration of bicarbonate to be added, two preliminary experiments were conducted in BOD bottles at two different initial DO levels.

Algal cultures were grown in medium containing different concentrations of  $\text{NaHCO}_3$ , varying from 0 to 500 mg/l, under light intensity of 80 ft-c. The DO and solid production indicated that a concentration of 100 mg/l would serve the purpose well.

Various workers have reported different media for growing Chlorella pyrenoidosa. Some of them, together with the medium used in the bioenvironmental laboratories of Oklahoma State University for activated sludge studies, were compared when fortified with 100 mg/l of bicarbonate. The composition of the modified media are given in Table II.

Six 4-liter volumetric flasks were used to grow the algal culture for this comparative study. One liter of each medium was used to which algal seed of 20 ml was added. The light intensity was 80 ft-c, provided by fluorescent lamps which were on at all times during the experiment. Samples were taken approximately once each twelve hours, and optical density was measured in Coleman (Model 6D) spectrophotometer at 540  $\text{m}\mu$ .

After 174 hours, the growth in media I and V was less than in the others, and they were discarded. In the second transplantation only media II, III, IV, and VI were tested. After 192 hours, the growth in media II and IV was lagging behind the other two (III, VI). Optical density measurement was continued for an additional 312 hours. The growth was far greater in medium VI. Another transplantation was made in media III and VI, and the growth was followed for



TABLE II

## COMPOSITION OF VARIOUS MEDIA TESTED

Constituents	Concentration - mg/l					
	I	II	III	IV	V	VI
$\text{NaHCO}_3$	100	100	100	100	100	100
$(\text{NH}_4)_2\text{SO}_4$	500	500	-	-	-	-
$(\text{NH}_4)\text{NO}_3$	-	-	1500	-	-	-
$(\text{NH}_4)\text{Cl}$	-	-	-	50	-	-
$\text{NaNO}_3$	-	-	-	1000	182	1000
$\text{K}_2\text{HPO}_4$	4280	4280	-	250	21.75	1000
$\text{KH}_2\text{PO}_4$	2108	2108	-	-	8.5	-
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	-	-	-	-	33.4	-
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	100	100	-	513	22.5	200
$\text{FeCl}_3$	0.5	1.5	-	3	0.15	-
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	-	-	-	-	-	50
$\text{K}_2\text{SO}_4$	-	-	1500	-	-	-
$\text{MnSO}_4$	10	10	-	-	-	-
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	-	-	-	-	-	2
$\text{CaCl}_2$	7.5	7.5	-	50	27.5	20
$\text{NaMoO}_4$	-	-	-	-	-	1
PH salt mixture-	-	-	1500	-	-	-
Tap water	100ml	100ml	100ml	100ml	100ml	100ml

Distilled water to make up to the required volume

- I. Medium used in bioenvironmental engineering laboratories for activated sludge units
- II. Modification of I;  $\text{FeCl}_3$  was increased to 1.5 mg/l from 0.5 mg/l
- III. Medium of Eyster, et al. used by Wu (81)
- IV. Allen's medium (51)
- V. Dilution water medium (51)
- VI. Medium recommended by Stanier, et al. (85).

287 hours. In this experiment, also, the growth was greater in medium VI, and medium VI was selected for use in further experiments.

#### D. Experimental Procedures

##### 1<sub>a</sub>. Continuous Flow Study with Algae and Bacteria (12 Hours Light, 12 Hours Dark)

Tanks C and D were used for this study; Figure 6 shows the experimental setup. A modified one-liter beaker was used as a constant head tank. A one-liter beaker was fitted with an outlet at the bottom on the side, and a glass tube of one inch diameter at the center on the bottom. The line from the feed bottle delivered medium to the annular space between the glass tube and the sides of the beaker. The outlet from the beaker served as the inlet for the reactor. When the feed level rose above the top of the central tube, the feed overflowed through the central opening to the waste collector. Thus, the head was always kept constant, causing an even flow irrespective of the level of the feed in the feed bottle. Lighting (overhead) was provided by thirteen GRO-LUX fluorescent lamps (Sylvania), 40 watts each, hung side by side. In addition to the reflectors in the fixtures, a sheet of cardboard wrapped with aluminum foil was placed on top of the fixtures. The light intensity at the top level of the reactor was between 425 and 475 ft-c. A 12-hours-on and 12-hours-off lighting cycle was employed.

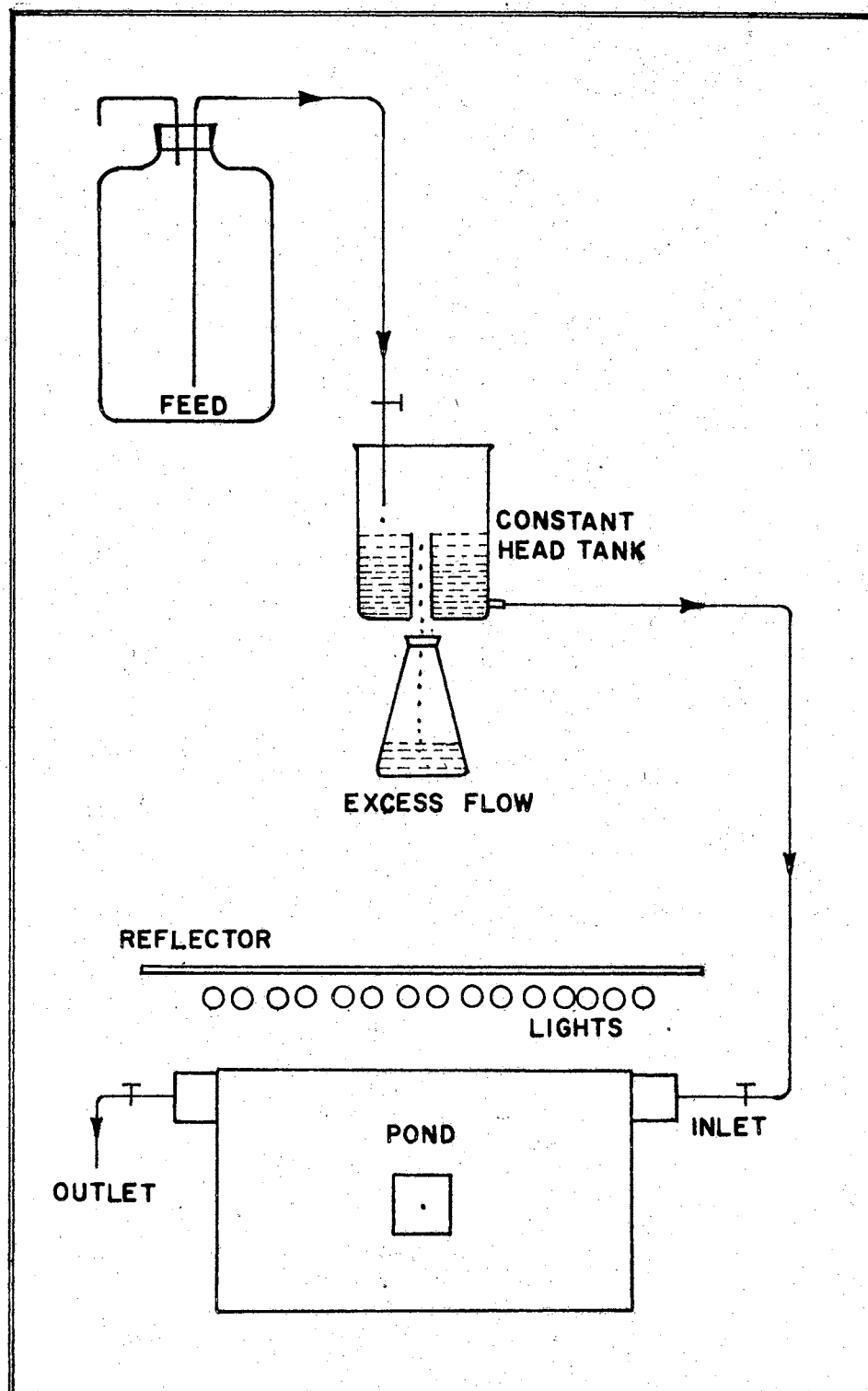


Figure 6 - Schematic diagram of experimental setup for continuous flow studies with algae.

The feed bottle, constant head tank, and the lines were autoclaved each time they were put into use. Generally, these were changed once in thirty-six hours. The effluent was collected and measured in order to have a check on the flow rate. Sewage and Chlorella pyrenoidosa were used as seed. After one week of operation, regular samples were taken at the beginning and end of the lighting period. Dissolved oxygen, solids, oxidation reduction potential, pH, and filtrate COD of the effluent were measured. The influent DO and COD were checked occasionally. The flow rate for a 10-day detention time in the closed system was 3.27 ml/min. At this rate it took about two hours to collect approximately 500 ml for DO determination. To avoid this problem, the rate of flow was increased during sampling. The valve in the outlet line was opened more to draw off more effluent. The decreased feed level in the constant head tank was brought to the rim of the central tube by withdrawing feed from the feed bottle.

Experiments were conducted with varying glucose concentrations with a detention time of ten days in two systems; i.e., one open to the atmosphere, and the other closed. A closed system with a detention time of twenty days and a glucose concentration of 500 mg/l was also studied.

#### 1<sub>b</sub>. Continuous Flow Study with Bacteria (No Algae Present)

Pond C was used for this study, which was conducted in the dark; settled sewage was used as seed. A Sigmamotor

pump was used to pump the feed to the influent side well. The detention period was ten days. Dissolved oxygen, pH, and COD of the effluent were determined twice daily. The effluent sample was taken from the exit side well of the pond. At times DO was measured for samples taken at the influent end and at the center of the main chamber. The reactor liquor was syphoned into standard 300 ml BOD bottles for determination of DO. Experiments were conducted with various glucose concentrations in the feed.

## 2. Physical Deaeration and Reaeration

Studies on physical reaeration were conducted in the dark, using the experimental ponds C and D. Both the rates of deaeration and reaeration were evaluated by conducting experiments with initial DO levels above and below the saturation values, using tap water at temperatures of  $22 \pm 2^{\circ}\text{C}$ .

### 2<sub>a</sub>. Deaeration

The rate of deaeration (stripping) was determined using water supersaturated with DO. The experimental pond was filled; pure oxygen was bubbled into the water through the port in the central well for about two hours. All openings were kept closed overnight. On the day of the experiment, four liters of the oxygenated water were drained through the central port into a bottle; then the exit port of the pond was opened and the extra water was drained off. The water level in the tank was the same as that of the medium in the tank for open system experiments.

An adjustable flow pump was used to circulate the water from one end well to the other end well to simulate the condition in the continuous flow experiments described earlier. The intake tube of the recirculation pump had two branches. One end was placed in one side well of the pond three inches below the water surface. The other end was placed in the bottle with oxygenated water three inches below the surface of the water in it. Other than at sampling times, the line from the bottle was closed. Whenever DO was determined by the chemical method, 500 ml of water was taken out of the pond. The same quantity of water from the bottle was pumped into the tank through the end of the line which was in the bottle.

The outlet tube of the pump ended in an airtight lucite cylinder. On the top of this lucite cylinder a DO probe was attached for electronic recording of DO, and a magnetic stirring bar was placed at the bottom of the cylinder. The cylinder assembly was placed on top of a magnetic stirrer. The effluent water from this cylinder was carried to the other side well of the pond, and it was let out three inches below the surface of the water.

Twice daily, DO concentrations were determined chemically. Standard 300 ml BOD bottles were used, and the water which was removed was replaced from the bottle, as mentioned previously. DO was recorded continuously, using a Galvanic cell oxygen analyzer and a Sargent recorder. The DO probe was calibrated before the start of each experiment, and the calibration was checked twice daily whenever DO was determined chemically. At the end of the experiment,

water from the pond was withdrawn into a 2-liter flask. The flask was thoroughly shaken, and the DO of the water was determined. The DO determinations were repeated daily until two consecutive readings were the same.

## 2<sub>b</sub>. Reaeration

The rate of reaeration (diffusion of oxygen from the atmosphere) was determined, using water devoid of oxygen. The experimental pond was filled; sodium sulfite in slight excess was added for chemical removal of the DO. Cobalt chloride (0.02 mg/l) (80) was used as a catalyst. The experimental setup and procedure were the same as described for the deaeration experiments.

## Biological Deoxygenation Studies

### 3<sub>a</sub>. Oxygen Utilization by Organotrophs

These studies were conducted in the dark and in the absence of algae. Experimental pond B was used for these experiments; BOD bottles were used for experiments which accompanied the work in the ponds. Samples were taken from one end of the tank through the valve arrangement previously described; an elevated distilled water reservoir was connected to the other end of the tank. Normally, both the inlet and outlet valves in the tank were kept closed. During sampling, both ports were opened and the required amount of sample was withdrawn. By this process an equal volume of distilled water replaced the sample volume. Then the ports were closed, keeping the system out of contact

with the atmosphere. Preliminary experiments indicated that the increase or decrease in the DO concentration due to the replacement was very small. In one experiment it ranged from 0.228 mg/l to 0.028 mg/l.

All normal precautions in running the BOD determinations were taken: scrupulous cleansing of bottles and pond, careful siphoning of samples, incubation in the dark, etc. The only omission was that the temperature was not precisely controlled; the bottles and pond were set up in a humid room in which the temperature range was  $25 \pm 2^{\circ}\text{C}$ .

#### Experiments in BOD Bottles

All constituents of the medium previously mentioned, excepting  $\text{NaHCO}_3$  and glucose, were mixed and made up to approximately 35 liters in a large carboy. Pure oxygen was passed through the medium for about three hours; then  $\text{NaHCO}_3$  and seed were added and mixed gently. Finally, the required amount of glucose was added, mixed gently, and the medium was carefully siphoned into BOD bottles (approximately ninety bottles were used). Almost one hour was required from the time of addition of substrate to complete filling of the BOD bottles; therefore, some change in initial DO was expected in different bottles. To minimize this effect, sampling was accomplished as follows: The bottles were arranged in the order in which they were filled. One from each end and another from the center of the row were taken for DO determination. Two more bottles were taken on different occasions from the center of the



above positions. Thus, it was expected that the variations in DO, if any, would be somewhat random.

### Experiments in Ponds

Making up the medium and seeding were done in the same way as described in the previous section. The sample volumes were measured each time in order to determine the volume displaced and consequently, the change in DO concentration which sampling might impose on the system. To keep the replacement at a minimum level, bottles of 150 ml capacity were used in place of standard BOD bottles (300 ml capacity).

### 3<sub>b</sub>. Oxygen Utilization by Photo-autotrophs

These experiments were conducted in a Warburg respirometer at a temperature of 25°C. The test organism, Chlorella Pyrenoidosa, was obtained from the American Type Culture Collection. It was grown on sporulation agar (86) and was transferred to a shaker flask (250 ml) and aerated for seven to ten days. Then 50 ml of this culture was used to seed the medium in a larger culture vessel. Air containing five per cent CO<sub>2</sub> was used as an additional carbon source. The CO<sub>2</sub> cylinder was connected to the culture vessel through a cotton trap. The intensity of the light was 300 ft-c.

### 3<sub>b<sub>1</sub></sub>. Heterotrophic Metabolism

Two-liter flasks were used as culture vessels. One liter of medium was used. The flask with the medium (devoid of carbonate, phosphate, and ferrous sulfate, which

were autoclaved separately and added), stopper, and the connections from the CO<sub>2</sub> cylinder were autoclaved. After cooling, 50 ml of seed from the shaker flask was added to the medium. The culture was used for experimentation after two weeks of growth.

Two Warburg flasks were set up with the seeded medium for measuring endogenous O<sub>2</sub> uptake. Glucose was added to the seeded medium to give a substrate concentration of 500 mg/l, and portions of it were placed in other Warburg flasks. Oxygen uptake was followed for forty-eight hours. Concentrations of the biological solids and COD and carbohydrate content of filtrates were determined initially and whenever the flasks were removed from the Warburg apparatus. Microscopic examinations were made to ensure that there was no bacterial contamination.

### 3<sub>b2</sub>. Endogenous Metabolism

Eight liters of medium were used in a 9.5-liter narrow-mouthed bottle for growing the algal culture. The autoclaving and seeding procedures were the same as described in the previous section. The culture was grown for fifteen to twenty days and used for experimentation.

On the day of the experiment, solids from 7.5 liters of the culture volume were harvested using a Sharples centrifuge. The centrifuge bowl, feed bottle, and all parts coming into contact with the algae were previously autoclaved. The harvested algae were re-suspended in autoclaved medium. Required volumes were placed in Warburg flasks,

and oxygen uptake was recorded for five to seven days. Duplicate flasks were taken off the Warburg apparatus at various intervals. Concentration of solids and COD of the filtrate were determined initially and whenever flasks were removed. Samples of the filtrates were subjected to gas chromatography in an attempt to detect metabolic products which might be excreted by the cells. Microscopic observations were made to check for contaminants.

#### E. Analytical Procedures

The experimental parameters examined and their methods of determination are given below.

##### 1. Dissolved Oxygen Concentration

Dissolved oxygen was determined chemically, as described in Standard Methods (87). The Alsterberg modification of the Winkler method was used. At times a galvanic cell oxygen analyzer (Precision Scientific Co.) was used to register DO electronically. A Sargent recorder was used for continuous reading.

##### 2. Oxygen Uptake

A Warburg respirometer, manufactured by the Gilson Medical Electronic Company was used for the measurement of oxygen uptake. Forty ml of culture fluid were used in the Warburg flasks of approximately 140 ml capacity. Experiments were run at a temperature of 25°C, and a shaker rate of 110 osc/min.

### 3. Biological Solids

The weight of biological solids was determined by the membrane filter technique.

### 4. Oxidation Reduction Potential (ORP) and pH

The pH was measured using a Beckman expanded scale pH meter. Oxidation reduction potential was measured by using a Beckman Zeromatic pH meter.

### 5. Chemical Oxygen Demand

The organic load was determined by using the COD test as described in Standard Methods (87). Both catalysts, silver sulfate and mercuric sulfate, were used. For lower concentrations, 0.025N dichromate, and for higher concentrations, 0.25N dichromate were used.

### 6. Carbohydrates

The anthrone test as described by Gaudy (88) was used to determine the carbohydrate concentration of the filtrate.

### 7. Glucose Concentration

The Glucostat test was used to determine the glucose concentration in the membrane filtrate. This was accomplished in accordance with the literature accompanying the enzyme sent by Worthington Biochemical Corporation (89).

## CHAPTER V

### RESULTS

#### 1. Continuous Flow Studies in Experimental Oxidation Ponds with Algae

Detention times employed in all continuous flow experiments are ten days, unless otherwise stated.

##### Mode of Presentation of Results

The general arrangement of the results of the studies with algae, with ponds open and closed to the atmosphere, is as follows: All parameters, observed and computed, are plotted against time. In the lower half of the figures the observed values of ORP and DO are plotted. The difference between the DO concentrations at the beginning and at the end of the light period was calculated and is shown on the figures as DO increase. Similarly, the difference between the DO concentrations at the beginning and at the end of the dark period was computed, and this is shown as DO decrease. The DO line is shown by a solid line during lighting, and by a dashed line during darkness. The light and dark periods are also shown. In the upper half of each figure the observed values of filtrate COD, biological solids, and pH are shown.

### Open Systems

Figure 7 shows the behavior of the open pond at an inflowing substrate concentration of 100 mg/l glucose, with a detention period of ten days. For the first fourteen days the DO concentrations averaged approximately 9.7 mg/l at the end of the light period, and the range was between 8.5 and 10.8. At the end of the dark period, the average value was 1.9 mg/l, with a range between 0.9 and 2.9 mg/l. The average change in DO concentration was 7.9 mg/l. The ORP gradually increased from 120 to 180 millivolts. The pH remained at the same level, i.e., around 7.1. The biological solids concentration exhibited a diurnal variation for the first seven days, increasing during the light period, and decreasing during the dark period. Following this, the solids concentration varied irregularly from approximately 110 to 60 mg/l. The filtrate COD fluctuated, but in general there was an increasing trend from about 110 mg/l to 140 mg/l.

Figure 8 shows the behavior of the open pond for a continuous loading of 150 mg/l of glucose. The average DO concentrations at the beginning and at the end of the light period were 0.4 and 4.7 mg/l. The ORP increased from 80 to 170 millivolts before dropping to 125 millivolts. The pH dropped slightly as the run progressed. After three days the biological solids concentration averaged approximately 92 mg/l. The effluent COD was approximately 90 mg/l.

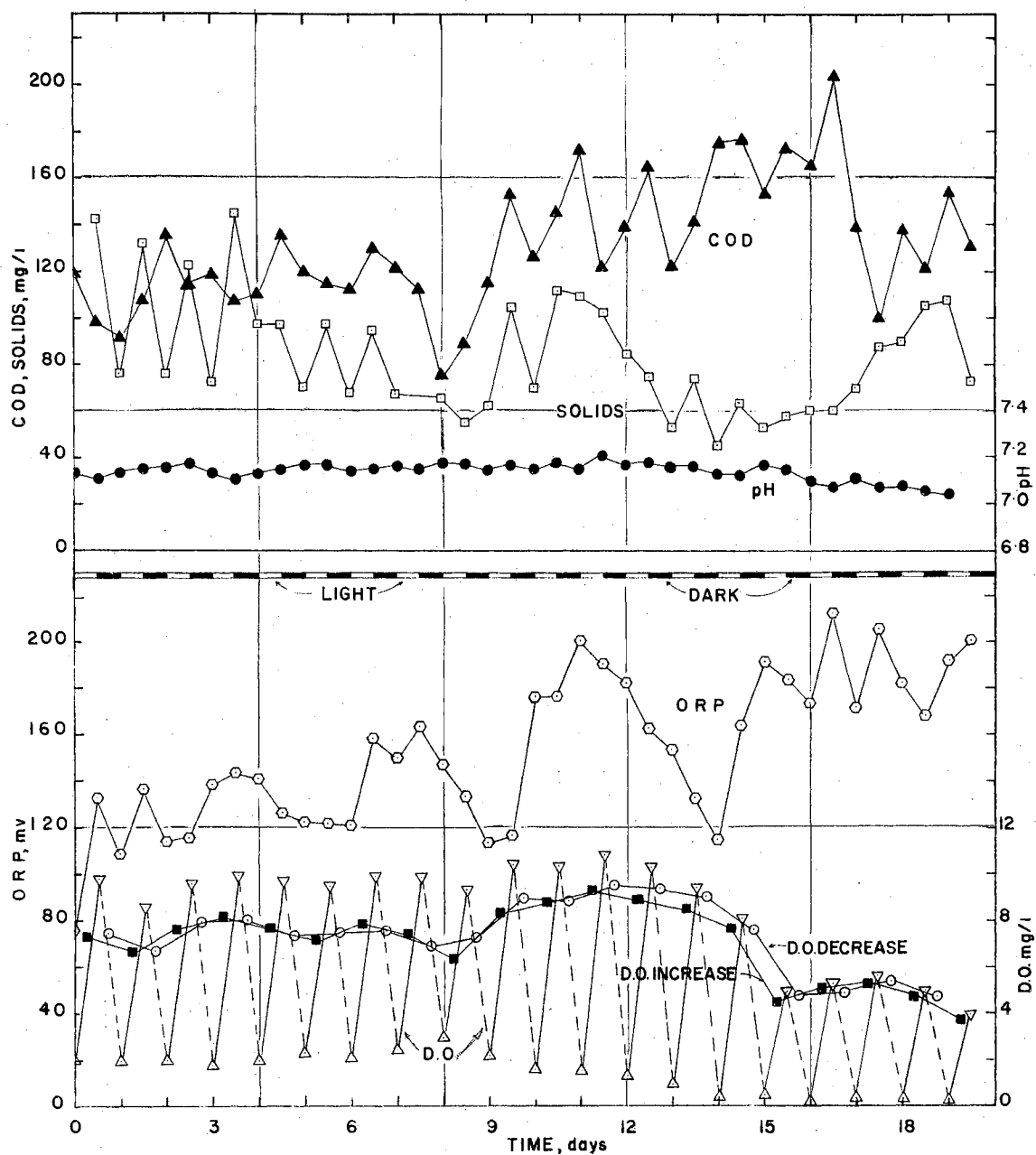


Figure 7 - Response of an open system to a continuous loading of 100 mg/l glucose.

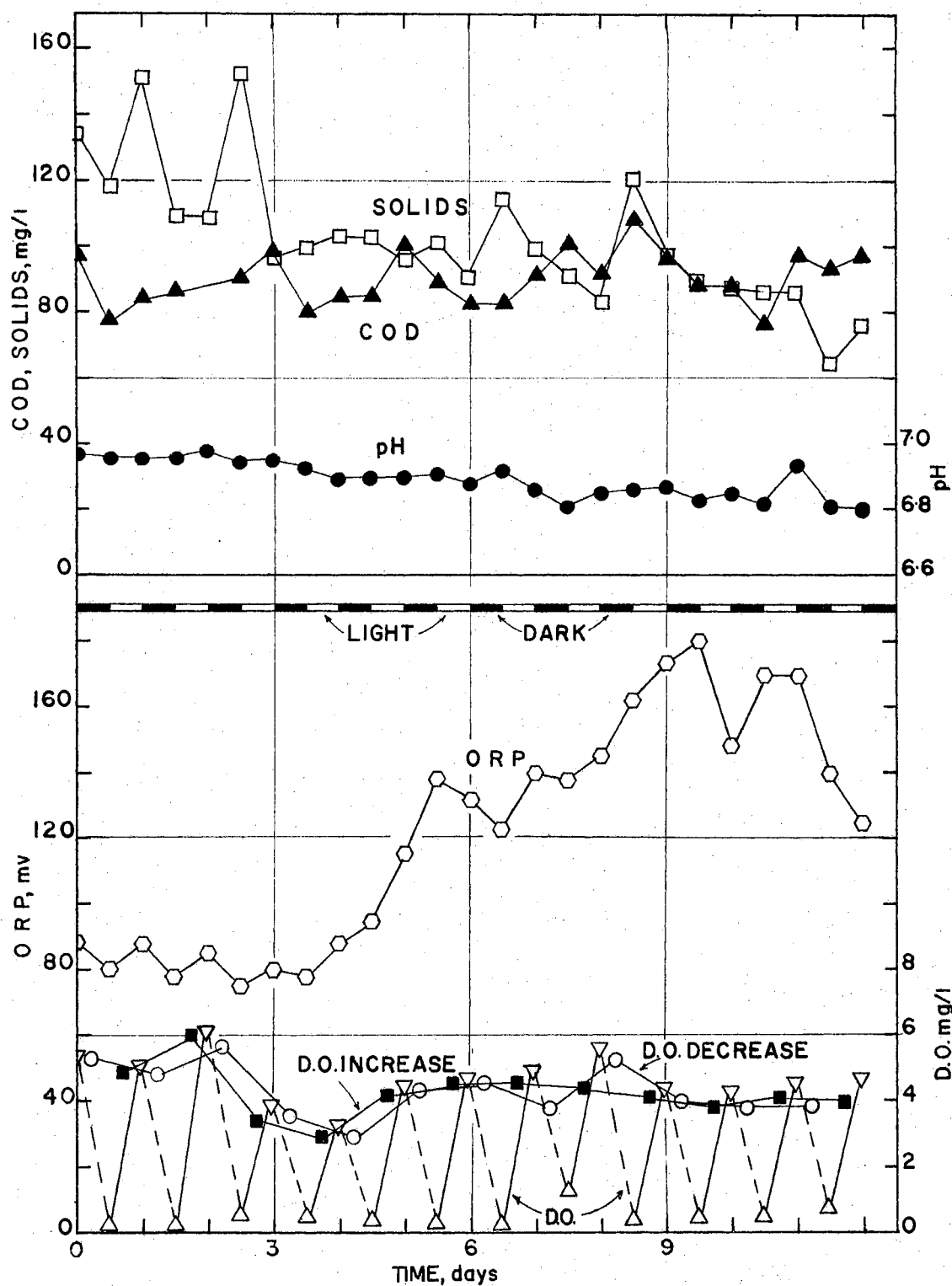


Figure 8 - Response of an open system to a continuous loading of 150 mg/l glucose.



Figure 9 shows the response of an open pond to a continuous loading of 250 mg/l of glucose. The DO at the end of the dark period was always zero, and all of the DO accumulated during the light period was removed during darkness. Therefore, both the DO increase and DO decrease are equal to the DO at the end of the light period. For this reason the DO curve was not plotted. Even though there was no DO at the end of the dark period, the DO at the end of the light period attained supersaturation levels for the first seven days (a maximum of 11.3 mg/l for one day). The changes in ORP were considerable (from +200 to -280 mv). After the third day, the ORP showed a diurnal change similar to the change in DO, increasing in the light and decreasing in the dark. The average increase during the light period was 210 millivolts, and the decrease during the dark period was 215 millivolts.

The biological solids concentration also fluctuated; most of the time it ranged between 40 mg/l and 90 mg/l. Only a slight pH change was noted. The COD was comparatively steady, gradually increasing from 60 to 75 mg/l. A comparative analysis and discussion of these systems follows in the next chapter.

### Closed Systems

Figures 10, 11, and 12 show the response of the closed pond for a continuous loading of 100 mg/l of glucose. The variations in the diurnal fluctuations of DO were small for the first nine days. After fourteen days and until the

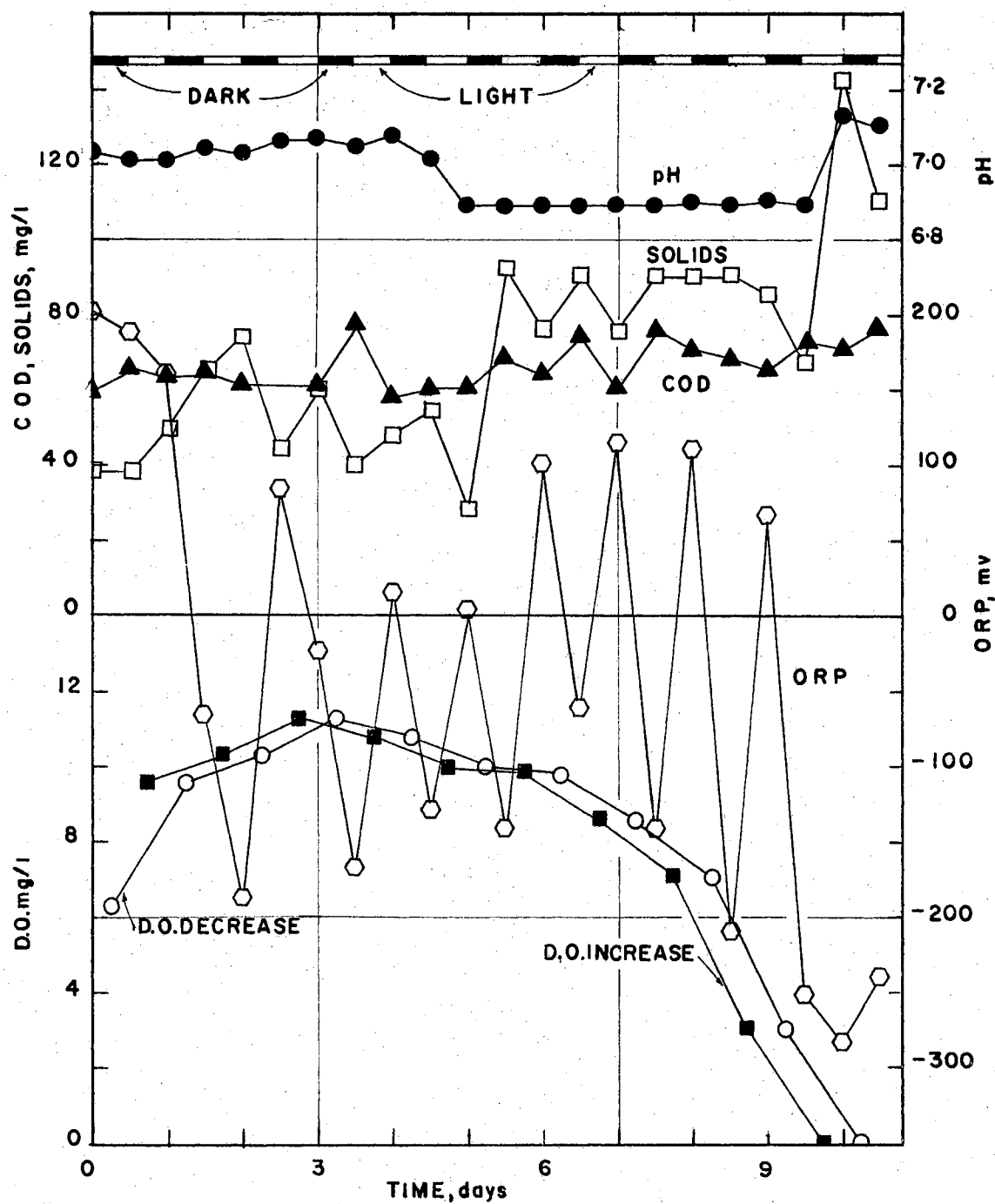


Figure 9 - Response of an open system to a continuous loading of 250 mg/l glucose.

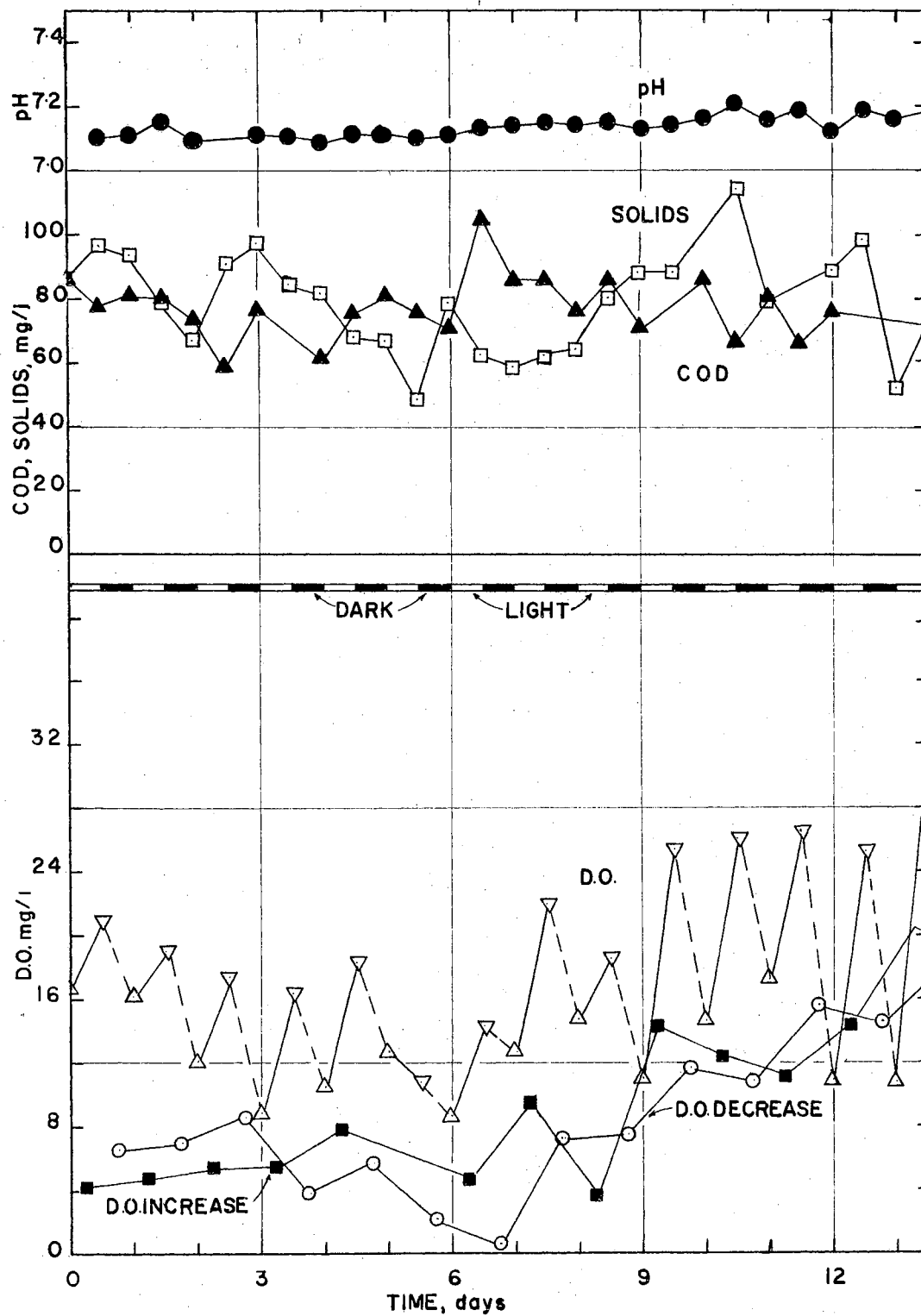


Figure 10 - Response of a closed system to a continuous loading of 100 mg/l glucose. (0-12 days).

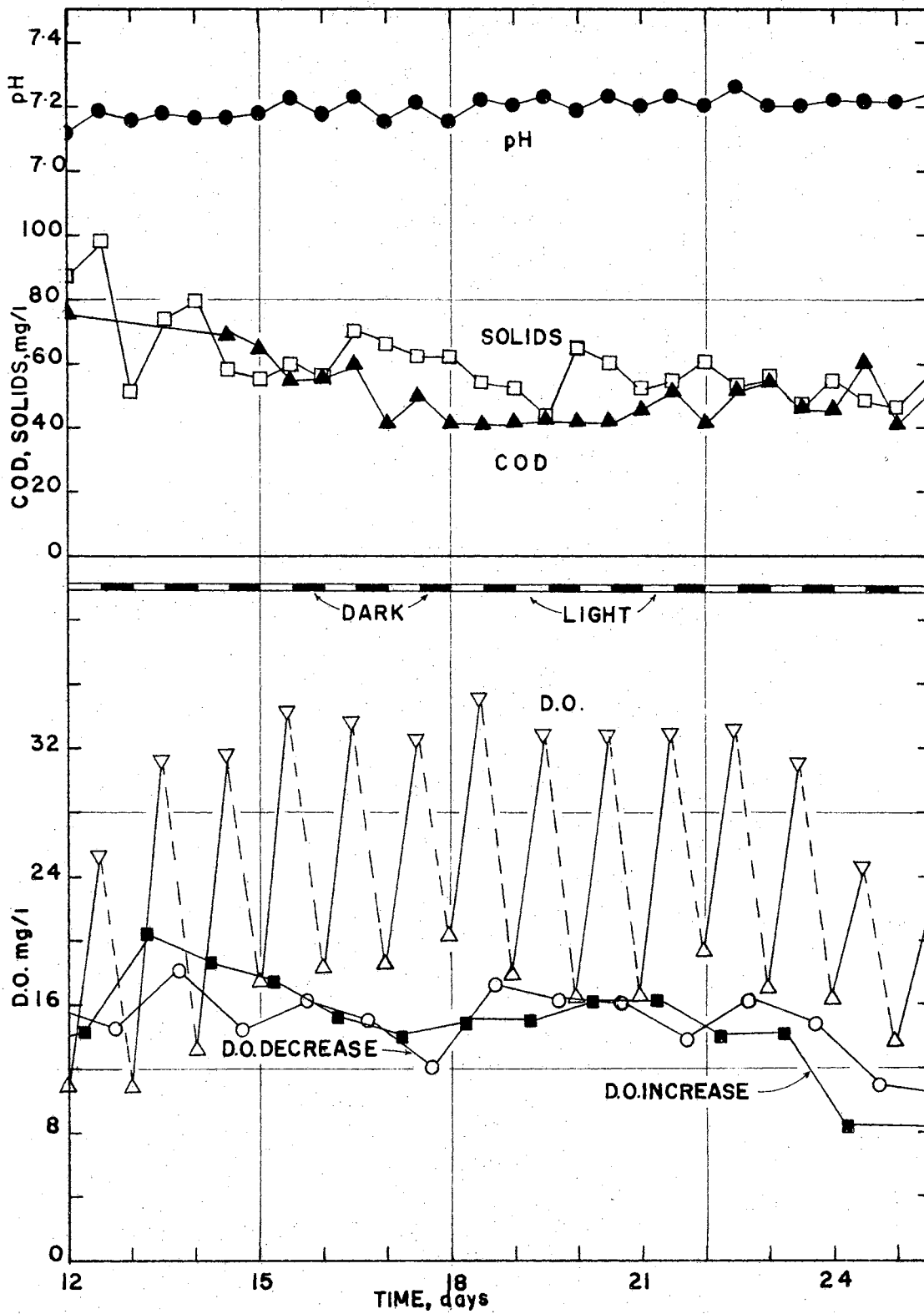


Figure 11 - Response of a closed system to a continuous loading of 100 mg/l glucose. (12-24 days)

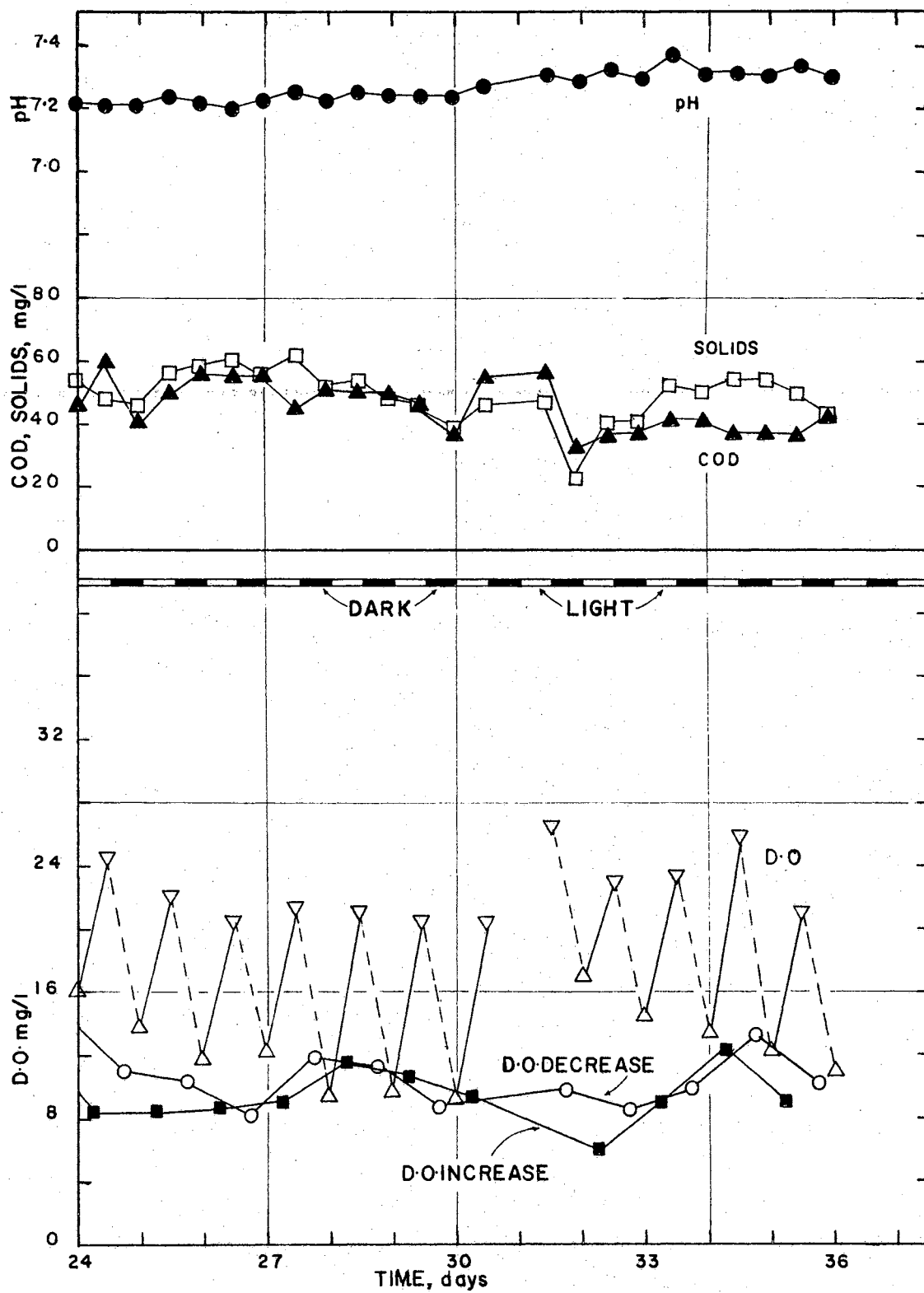


Figure 12 - Response of a closed system to a continuous loading of 100 mg/l glucose. (24-36 days)

twenty-third day, the system approached a condition of "balanced operation." During this nine-day period the average maximum DO was 33.1 mg/l, with a range of  $\pm 2.0$ . The minimum DO concentrations ranged between 16.6 and 20.4 mg/l, and the average was 18.0 mg/l. The average change in DO during this period amounted to 15.2 mg/l. The pond attained another "balanced condition" from the twenty-fourth to the thirty-sixth day of operation. The DO during the last twelve days was slightly lower, compared to the previous days. The maximum DO was in the range of 20.5 to 26.7 mg/l, with an average of 22.6. The minimum DO was in the range of 9.5 to 16.9 mg/l, with an average of 12.8 mg/l. The average amplitude during this period was 9.8 mg/l. Regarding pH, there was a slight rise (approximately 0.25 units) as the run progressed. During some days the pH varied diurnally, increasing about 0.05 units in the light, and decreasing by about the same amount in the dark. The solids showed marked oscillations for fourteen days, after which they were almost steady. The average solids concentration between the fourteenth and the twenty-third day was 57 mg/l. During the last ten days the average solids concentration was reduced to about 50 mg/l. The filtrate COD stabilized after twelve days. The average COD during the first "balanced operation" was 48 mg/l, which was reduced to about 45 mg/l during the second "balanced period."

Figures 13 and 14 show the response of a closed pond to a continuous loading of 150 mg/l of glucose. As in the

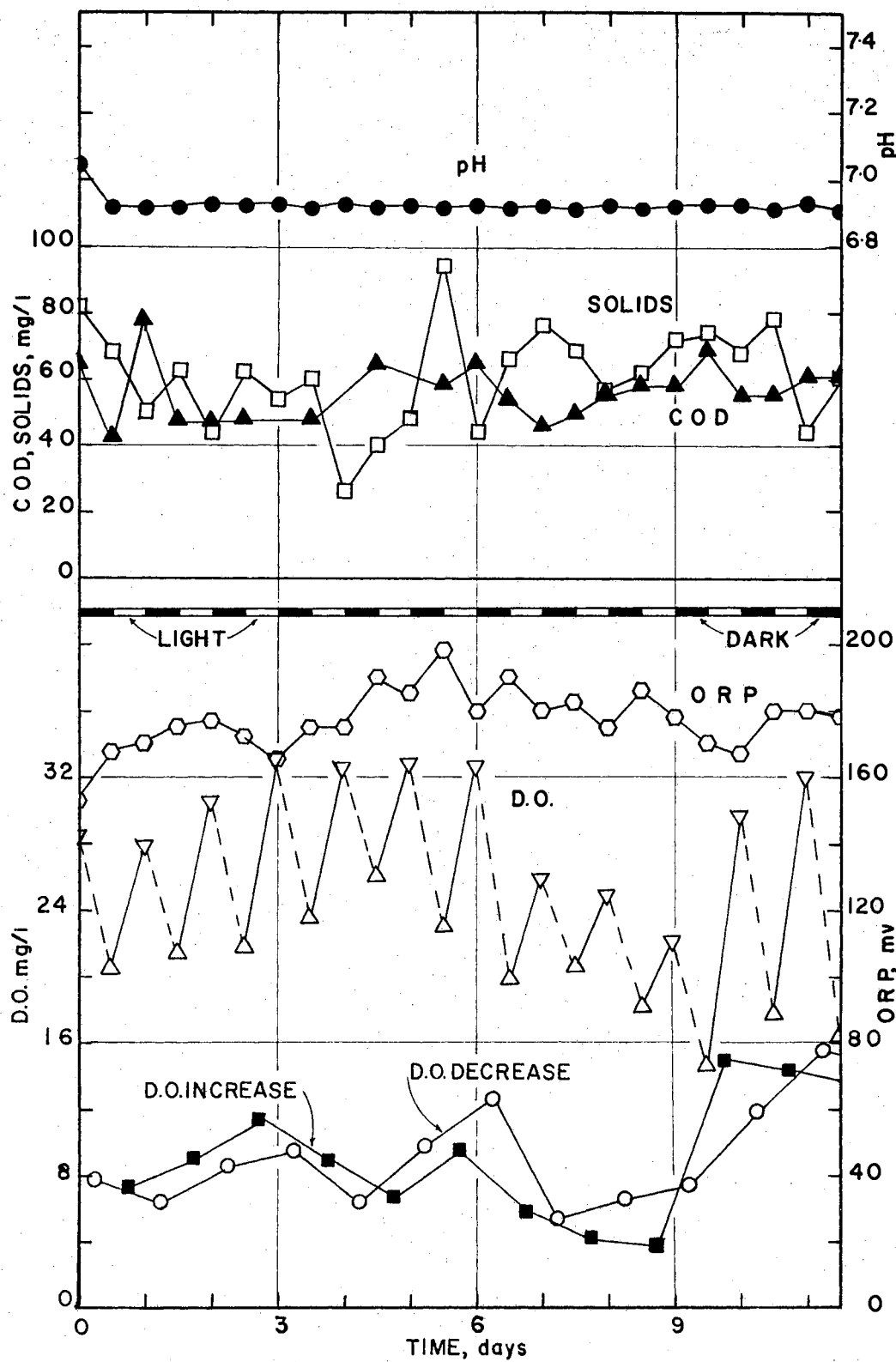


Figure 13 - Response of a closed system to a continuous loading of 150 mg/l glucose. (0-10 days)

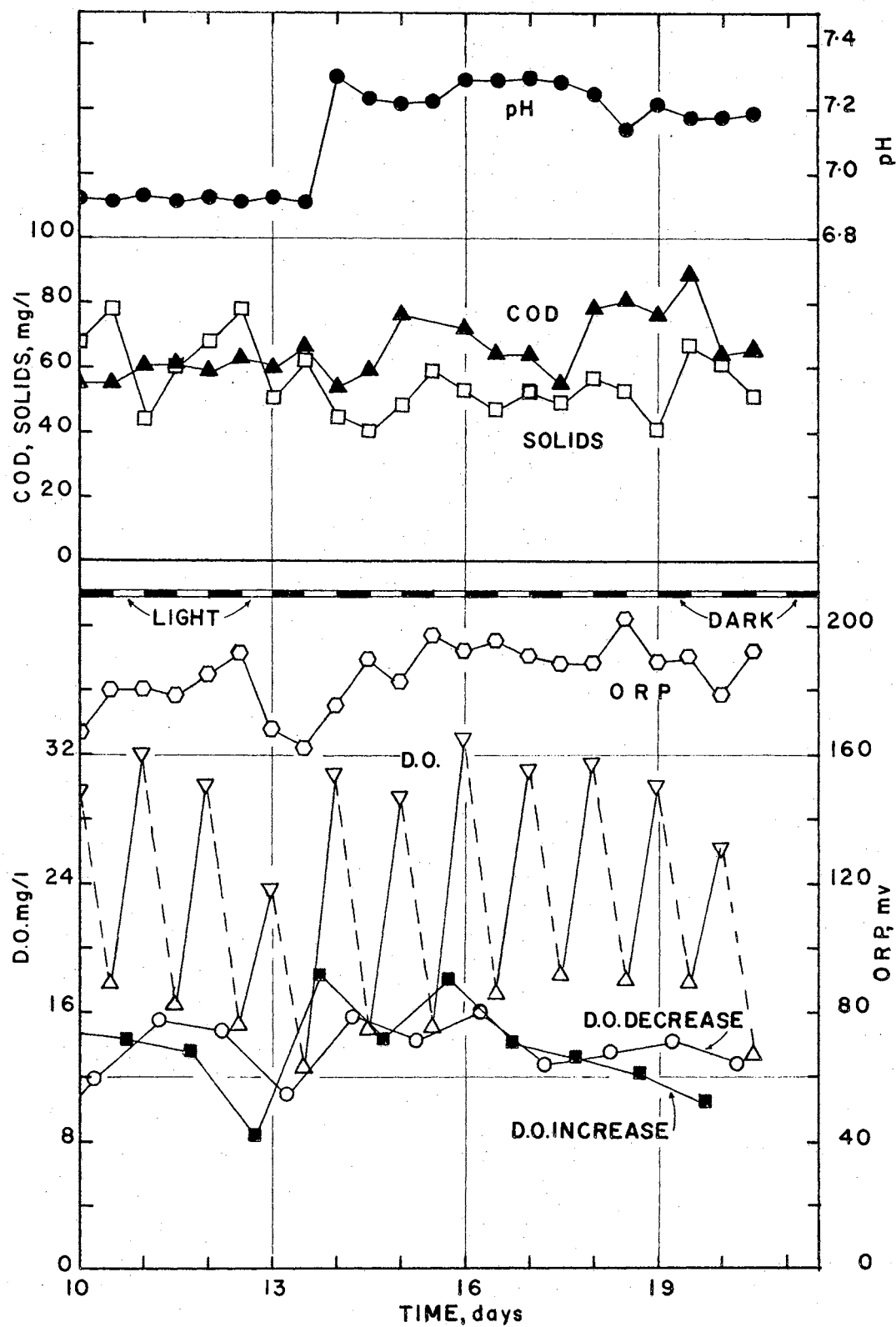


Figure 14 - Response of a closed system to a continuous loading of 150 mg/l glucose. (10-20 days)



previous case, the diurnal fluctuations were small for the first ten days. During the second ten days the DO attained a somewhat steady condition, i.e., it oscillated between approximately the same limits. The average maximum DO was 30.1 mg/l, and the average minimum DO was 16.0 mg/l. The average change was 14.1 mg/l; the ORP increased gradually from +153 to +185 millivolts in five days and, for the most part, remained at this level.

The pH remained constant for the first thirteen days at 6.9, and on the fourteenth day rose sharply to 7.3. The biological solids concentration was approximately 60 mg/l throughout the experimental period. The effluent COD varied between 50 and 80 mg/l, with an average of 65 mg/l.

Figures 15 and 16 show the response of a closed pond to a continuous loading of 250 mg/l of glucose. The DO at the end of the light period was approximately 35 mg/l for the first nine days of operation, and dropped to approximately 29 mg/l for the next three days. During this period the DO increase and the DO decrease in the same day were more or less equal, although the magnitude of the change dropped from an average of 29 mg/l during the first nine days to 14 mg/l during the last three days. Thereafter, the DO decrease remained at approximately 13.5 mg/l, whereas the DO increase was only 8.5 mg/l. As a result, the maximum and minimum DO followed a decreasing trend, and the DO dropped to zero during the dark period after eighteen days. The overall decrease in pH was very small,

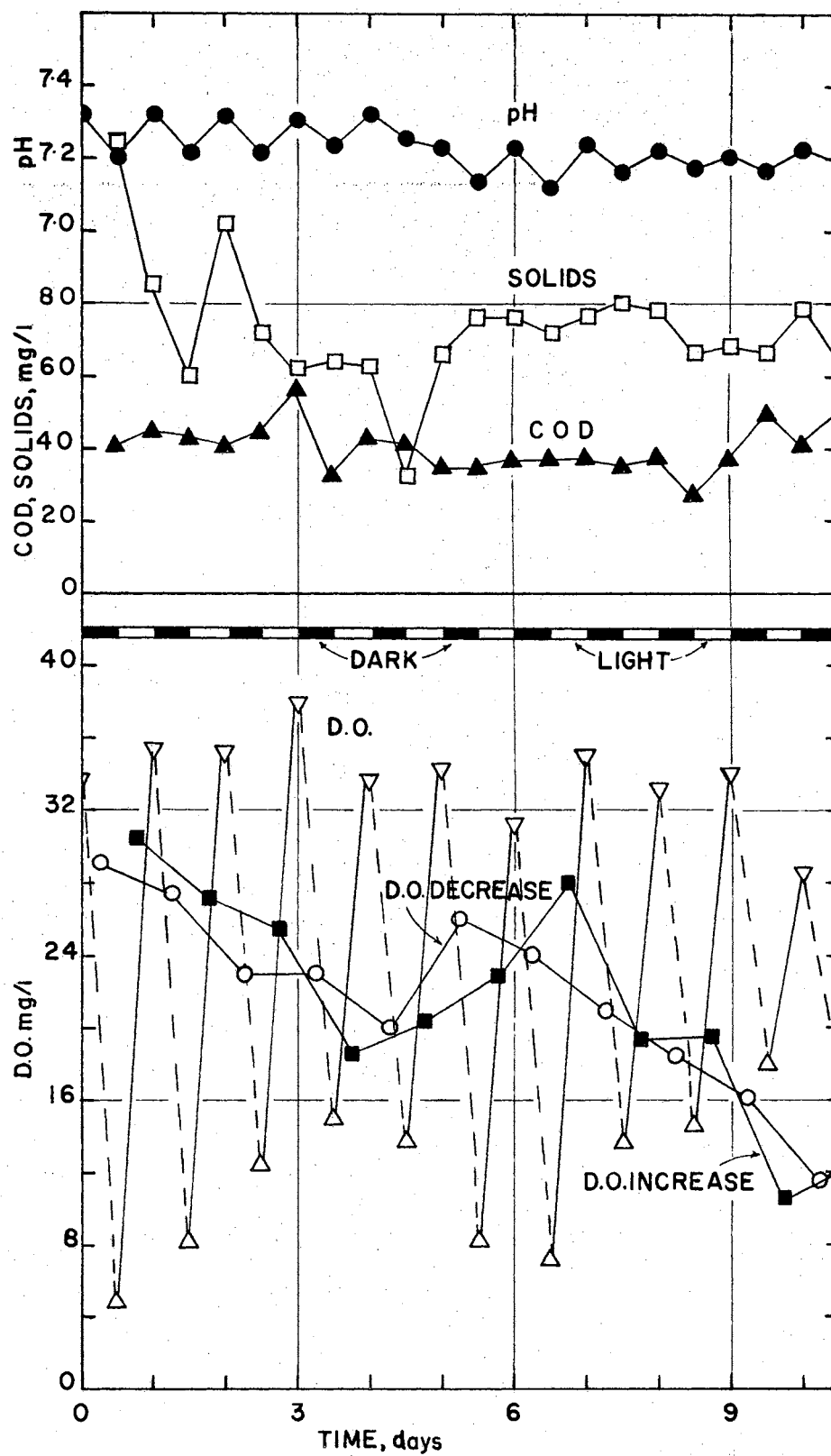


Figure 15 - Response of a closed system to a continuous loading of 250 mg/l glucose. (0-10 days)

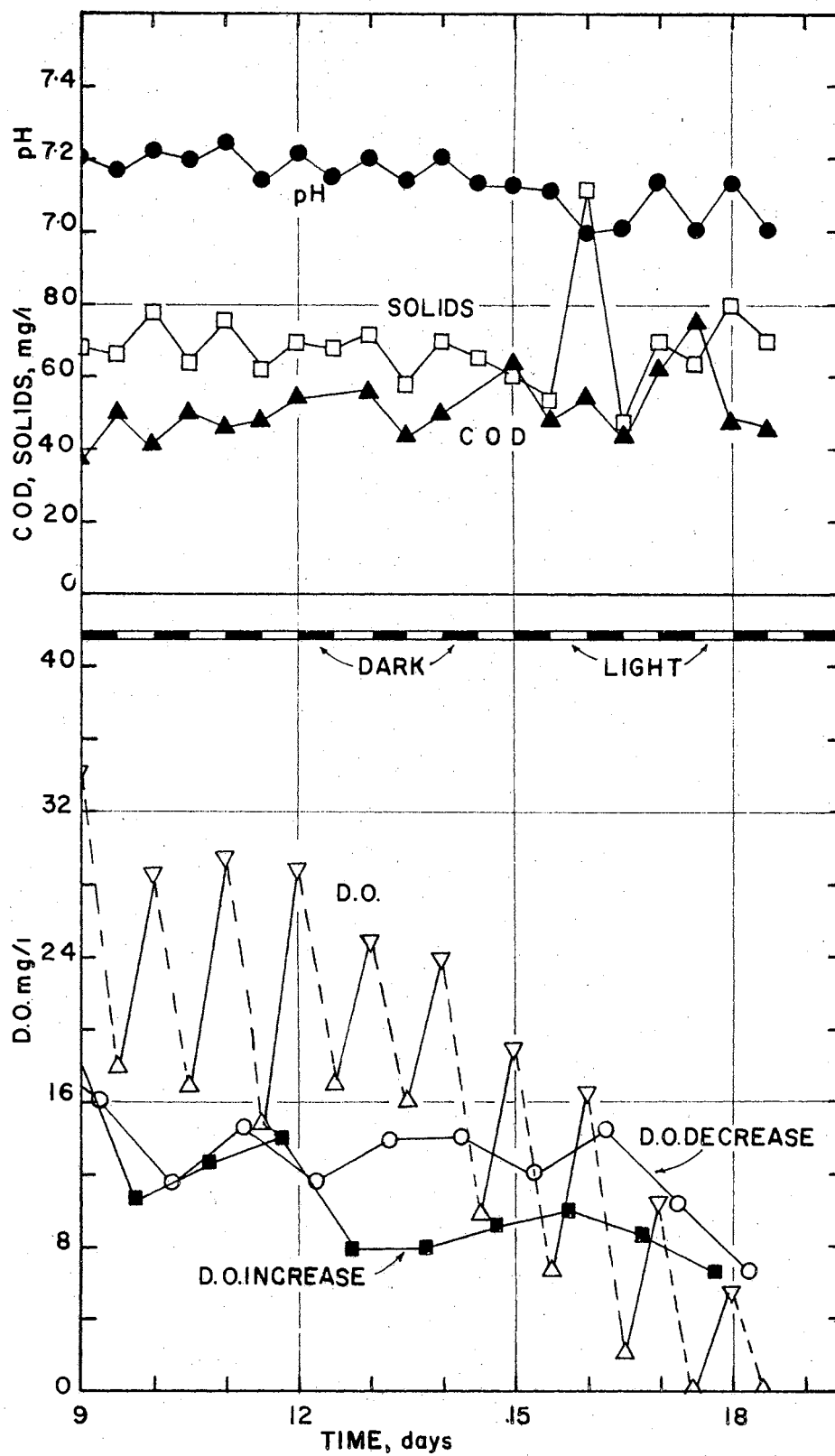


Figure 16 - Response of a closed system to a continuous loading of 250 mg/l glucose. (9-18 days)

amounting to about 0.18 units. However, pH followed a pattern of diurnal variation, increasing during the light period and decreasing during the dark period. Out of eighteen light periods, pH increased during fifteen, the increment ranging from 0.12 to 0.03. In nineteen dark periods pH decreased in eighteen, the decrease ranging from 0.12 to 0.02. The solids concentration ranged between 60 and 80 mg/l. The effluent COD (filtrate) averaged approximately 40 mg/l.

When the DO in the pond reached zero, an attempt was made to regenerate the system. For three days the lights were left on. For the following 3-day period the lights were on for fifteen hours in each twenty-four hours, i.e., the duration of dark periods was only nine hours. On the seventh day the system was brought back to the original light cycle of 12-hours-on, 12-hours-off. Figure 17 shows the performance of the regenerated system after the regular light schedule of 12-hours-on and 12-hours-off was adopted. The DO at the end of the light period ranged from 10.8 to 18.6 mg/l, averaging 13.9 mg/l. At the end of the dark period, some DO was present; the average DO was slightly in excess of 0.3 mg/l. Although the ORP dropped during the fifth day of operation, it averaged approximately 195 millivolts. The pH remained steady around 7.0. In this system, also, the pH showed, in general, a regular diurnal pattern of increase during light periods and decrease during dark periods; however, the maximum increase was very

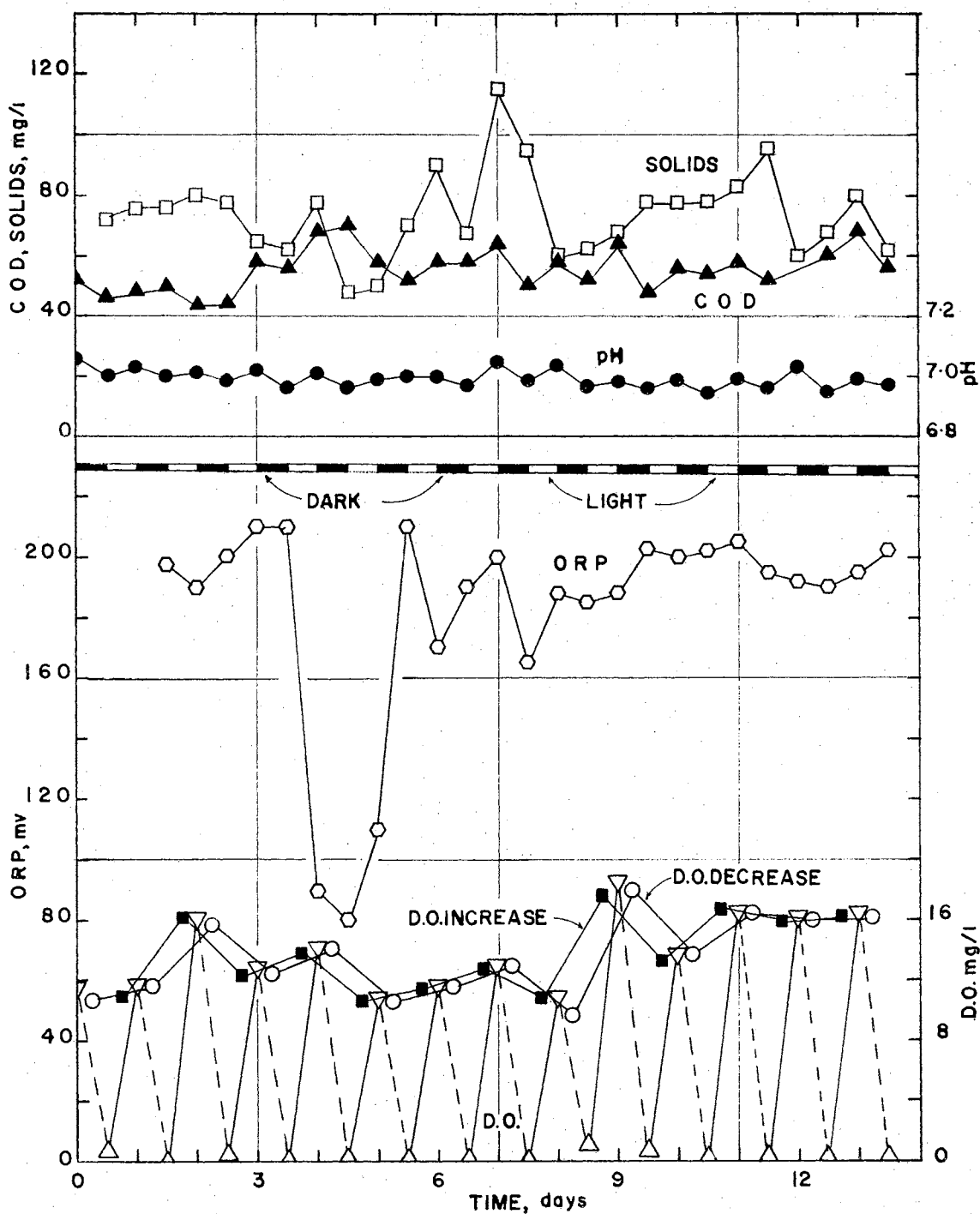


Figure 17 - Response of a "regenerated" closed system to a continuous loading of 250 mg/l glucose.

small. The solids concentration ranged between 60 and 80 mg/l, and the filtrate COD was  $55 \pm 10$  mg/l during most of the operational period.

Figure 18 shows the performance of a closed pond when subjected to a continuous loading of 500 mg/l glucose with a detention period of twenty days. No dissolved oxygen was detected during the experiment; the pH remained steady at about 6.9. The ORP was always negative (average value -310 millivolts). The solids showed a downward trend, while the COD decreased for the first two and one-half days and then increased. Since the system was anaerobic throughout the monitored period, observation was not continued.

## 2. Continuous Flow Studies in Open System Without Algae

Figure 19 shows the response of the open pond without algae to an incoming glucose concentration of 50 mg/l. At the beginning the pond was filled with medium containing substrate (50 mg/l) to the operating volume. Acclimated seed, started from settled sewage, was added in an amount of 5 ml/l, and the influent feed was pumped into the pond. The detention time was ten days. The dissolved oxygen in the effluent decreased to the zero level after two days, and the effluent was devoid of DO until the fourth day of operation. The increase in the effluent DO was very small for five and one-half days; thereafter the increase was gradual up to the tenth day, when it reached 3.6 mg/l. Beyond the twelfth day of operation the effluent DO remained almost at the same level (approximately 3.7 mg/l). The DO

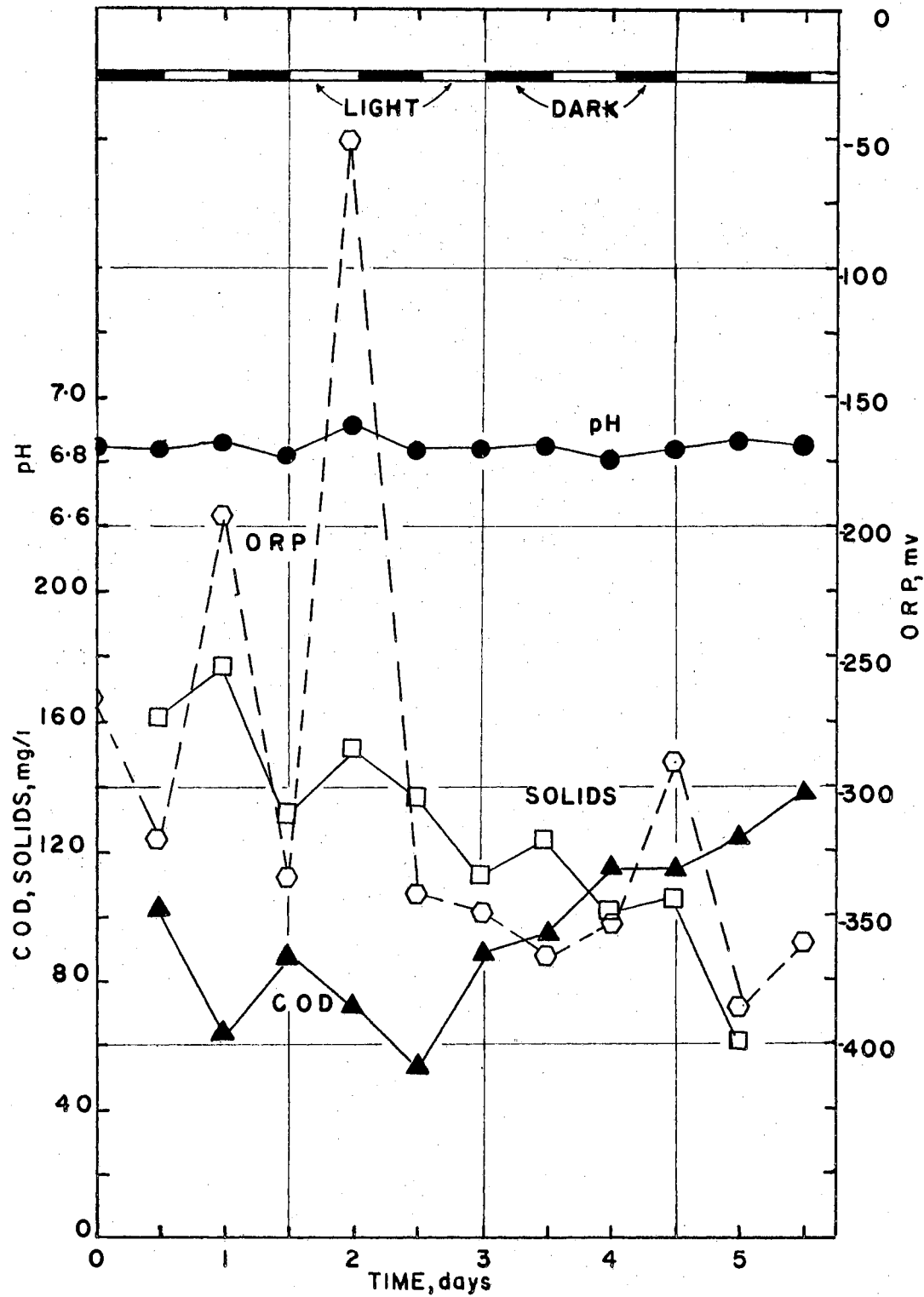


Figure 18 - Response of a closed system to a continuous loading of 500 mg/l glucose; detention period 20 days.

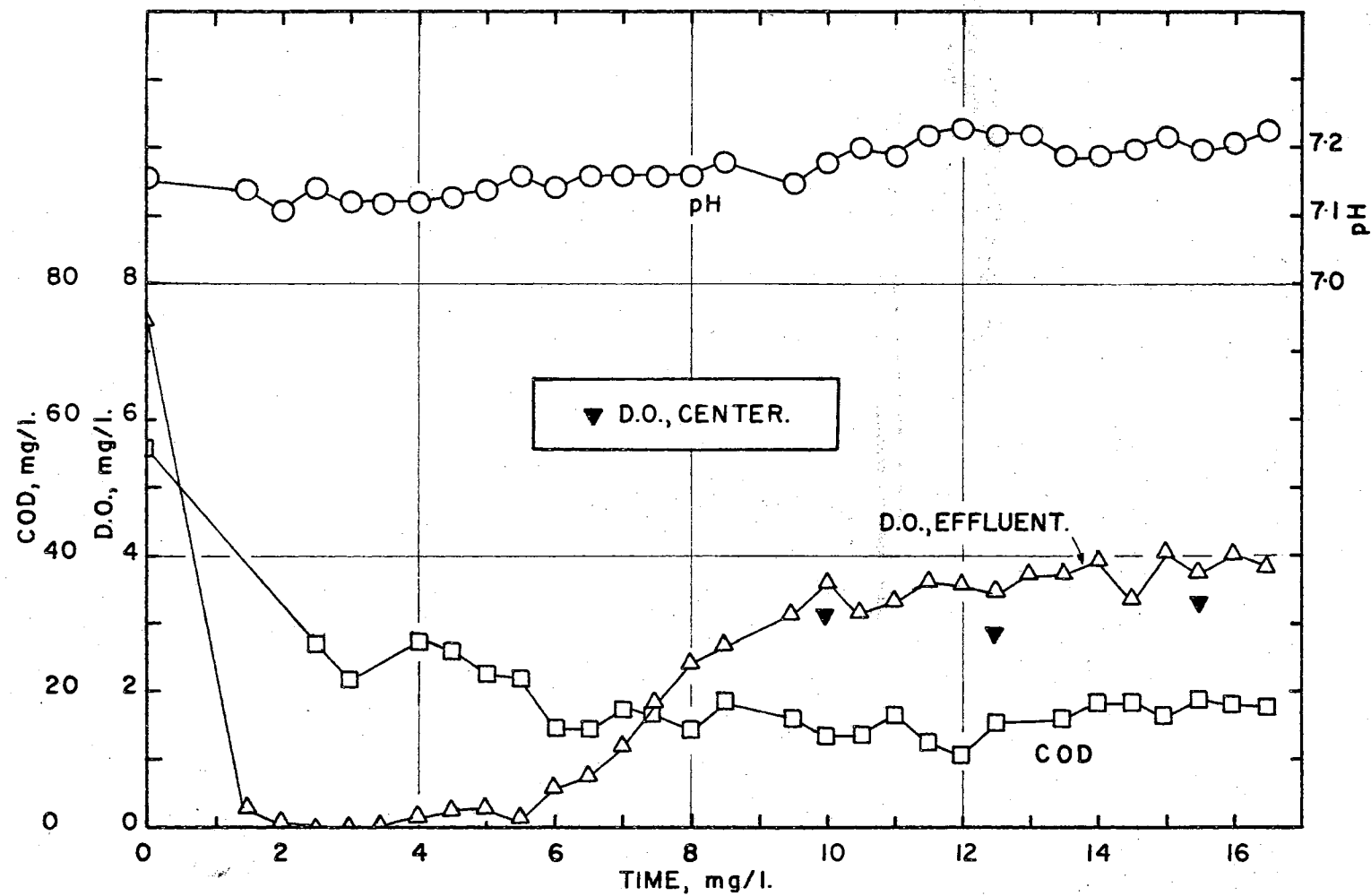


Figure 19 - Response of an open system without algae to a continuous loading of 50 mg/l glucose.



concentrations from samples at the center of the pond (three inches below the surface) were determined three times after the system recovered. They were approximately 0.5 mg/l lower than the corresponding concentrations in the effluent; the average influent COD was 56 mg/l. The effluent COD decreased sharply for three days and then decreased gradually up to the sixth day. After the sixth day the effluent COD remained steady at an average value of 17 mg/l; the removal was about 70 per cent. Only a very small increase in pH was noted.

Figure 20 shows the behavior of the pond when it received a glucose concentration of 80 mg/l. These results were obtained after changing the feed concentration from 50 to 80 mg/l glucose in the system shown in the previous figure. The effluent DO (3.8 mg/l) remained the same for thirty-six hours after the increase in the substrate concentration, and decreased to 3 mg/l during the next twenty-four hours. Thereafter it remained at approximately 3 mg/l for the remainder of the operational period, whereas the DO at the center of the pond was approximately 2 mg/l. The average DO at the influent end of the main chamber was approximately 0.3 mg/l. The effluent COD remained at the initial level of 17 mg/l for about five days, then increased gradually to approximately 20 mg/l the next day. Beyond this time, the effluent COD was more or less constant at approximately 20 mg/l. The average influent COD was 81 mg/l; the removal was approximately 75 per cent. There

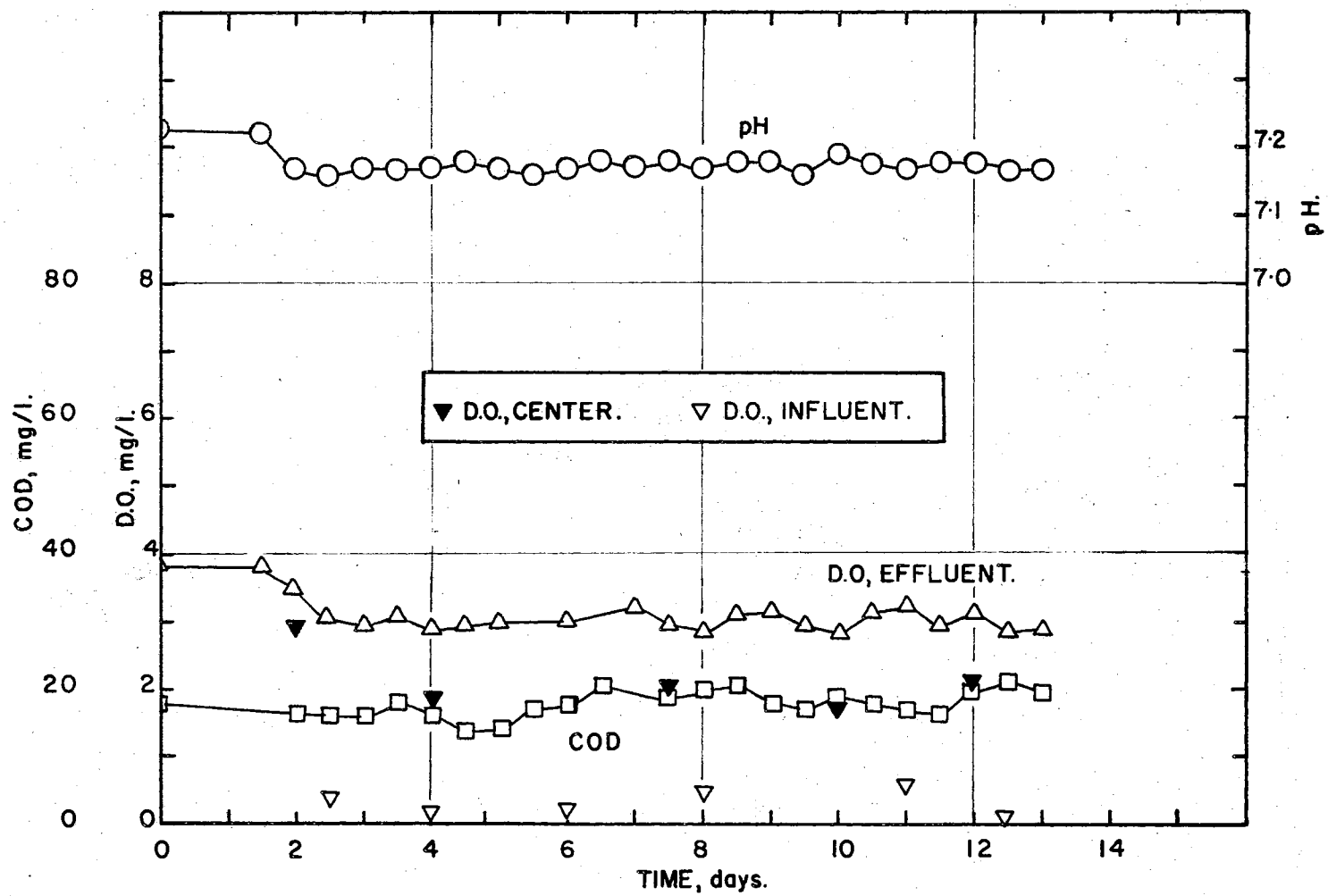


Figure 20 - Response of an open system without algae to a continuous loading of 80 mg/l glucose.

was a very small decrease in pH (0.05 units) during the first two days; thereafter the pH was steady throughout the experiment.

The response of the system to a continuous loading of 120 mg/l glucose is shown in Figure 21. This was also a continuation of the previous system. The substrate concentration was increased from 80 mg/l glucose to 120 mg/l; the figure shows the performance of the system after the increase. The effluent DO was reduced from approximately 3 mg/l to 0.5 mg/l in four days. The DO concentration increased to about 1.5 mg/l during the next three days, and once again decreased. From the tenth day onward the effluent DO was near 1 mg/l. The DO concentrations at the center of the pond were zero for all of the days tested, except that a trace amount was recorded for one day. Typical anaerobic conditions existed in the side well receiving the influent, as evidenced by the production of hydrogen sulfide and the black color of the reaction liquor. The settled sludge in the side well was black in color, and there was an accumulation of settled solids (approximately 3/4 cm) in the side well of an area 10 cm x 10 cm. The effluent COD increased to 29 mg/l on the fifth day, an increase of approximately 10 mg/l from the initial effluent COD. The COD curve was not "steady," in comparison with the previous systems, and averaged approximately 26 mg/l. The average influent COD was 133 mg/l; the pH remained more or less at the same level.

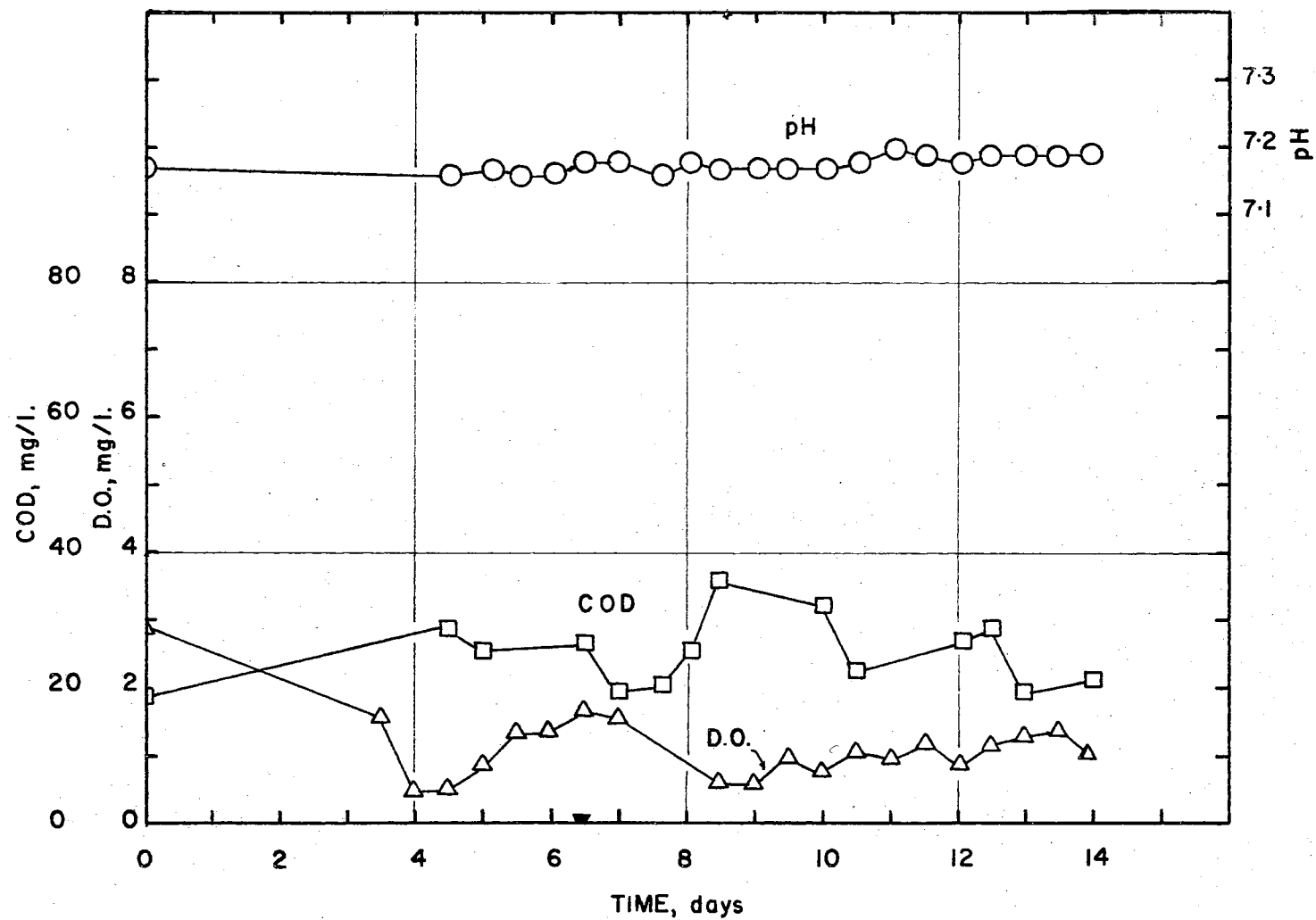


Figure 21 - Response of an open system without algae to a continuous loading of 120 mg/l glucose.

### 3. Physical Oxygenation and Deoxygenation Characteristics of the Experimental Ponds

#### Mode of Presentation of Results

The observed values of the DO concentrations for each experiment are plotted against time in the lower half of each figure on arithmetic paper. At the end of each experiment the saturation values of the DO concentration were determined (observed values). The differences between these values and the observed values of the DO concentration at various time, i.e, deficits or excesses, were computed. These deficits or excesses were plotted against time in the upper half of each figure on semilogarithmic paper with time on the arithmetic scale. The slopes of the lines in the upper half of the figures were calculated; these values represent the reaeration or deaeration rate constants. The saturation values for DO concentration were also obtained by calculation from the DO-time curve, using the  $\alpha$  method (81) as described previously. Deficits or excesses were computed based on these values; using these differences in DO concentrations, the rates of reaeration and deaeration were also determined.

#### A. Deaeration

Figure 22 shows the course of deaeration in Pond C. The observed saturation value was 8.32 mg/l, and the deaeration rate was  $0.305 \text{ day}^{-1}$  ( $0.0127 \text{ hr}^{-1}$ ). The computed values were 7.81, 0.292, and 0.0122, respectively. Figure 23 shows the deaeration in pond D. The observed

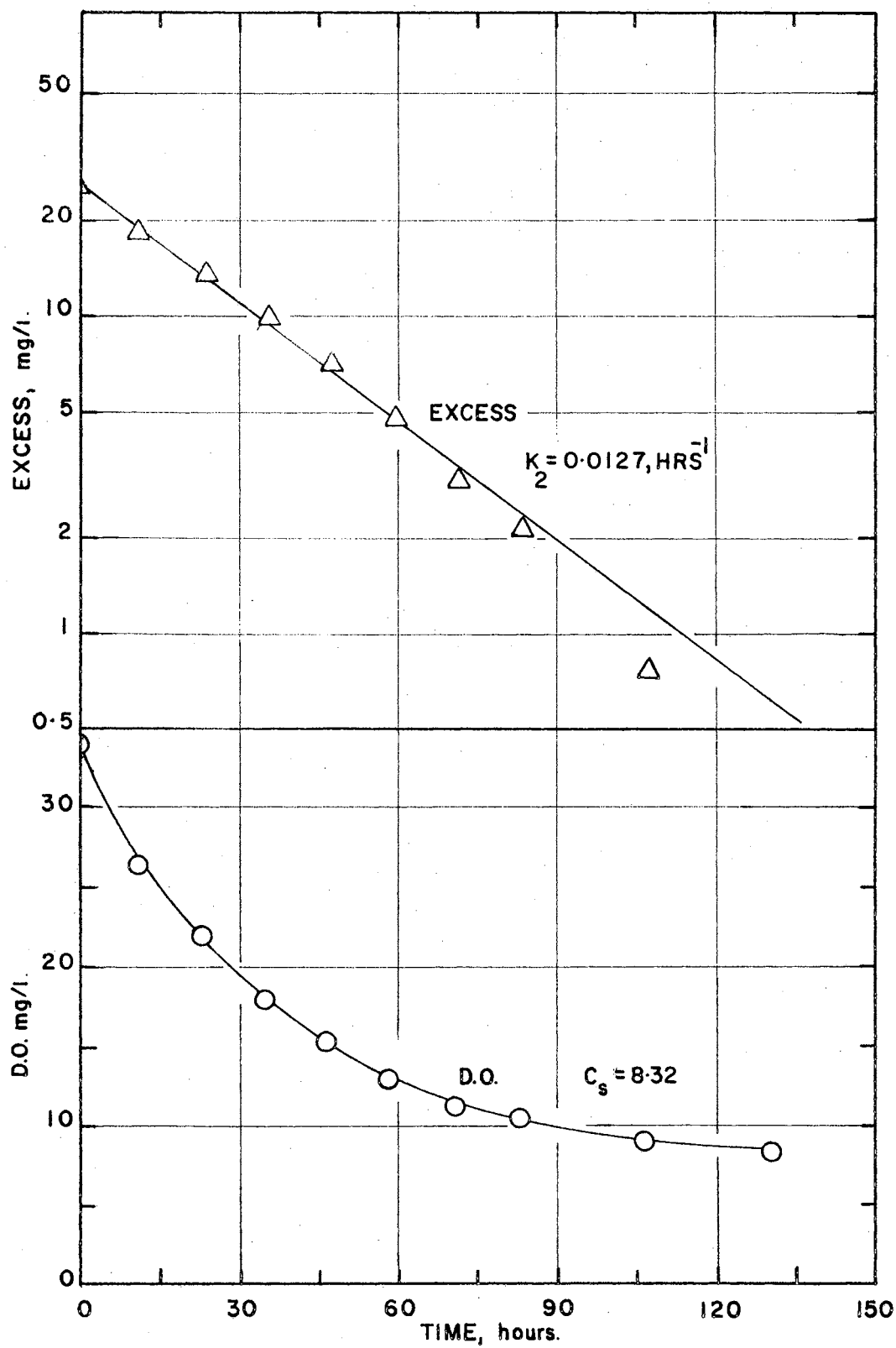


Figure 22 - Deaeration characteristics of Pond C.

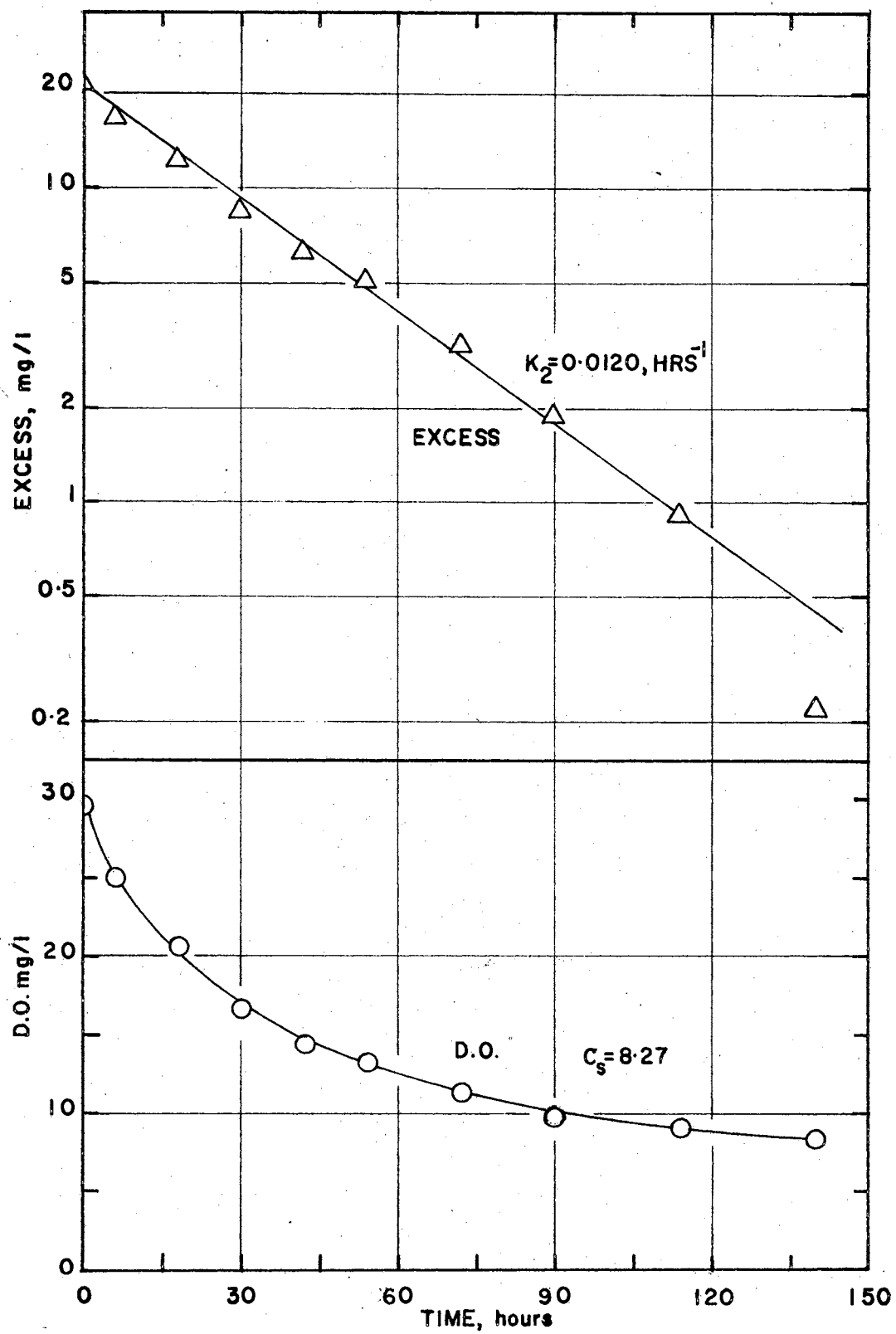


Figure 23 - Deaeration characteristics of Pond D.

saturation value was 8.27 mg/l, and the  $K_2$  rate based thereon was  $0.288 \text{ day}^{-1}$  ( $0.0120 \text{ hr}^{-1}$ ). The computed saturation value was 8.21 mg/l, and the  $K_2$  rate based on this value was  $0.282 \text{ day}^{-1}$  ( $0.0118 \text{ hr}^{-1}$ ).

#### B. Reaeration

Figure 24 shows the reaeration in pond C, the pond without baffles. The observed saturation value of the DO concentration was 8.15, and the value computed by the  $\alpha$  method was 8.26 mg/l. The corresponding reaeration rates were  $0.298 \text{ day}^{-1}$  ( $0.124 \text{ hr}^{-1}$ ), and  $0.290 \text{ day}^{-1}$  ( $0.0121 \text{ hr}^{-1}$ ).

Figure 25 shows the reaeration in pond D, the pond with baffles. The observed and computed saturation values of DO were 8.18 and 8.14 mg/l, respectively. The corresponding reaeration rates were  $0.308 \text{ day}^{-1}$  ( $0.0128 \text{ hr}^{-1}$ ) and  $0.316 \text{ day}^{-1}$  ( $0.0132 \text{ hr}^{-1}$ ).

### 4. Biological Deoxygenation Due to Organotrophic and Photo-autotrophic Organisms

#### A. Oxygen Utilization by Organotrophs

##### Mode of Presentation

For each substrate level the results for two typical experiments are shown. The DO concentrations at various times, both for BOD bottle systems and for the pond are shown. The oxygen uptake values at various times were calculated by deducting the DO concentrations at those times from the initial DO concentration, and these calculated values are plotted with respect to time for both



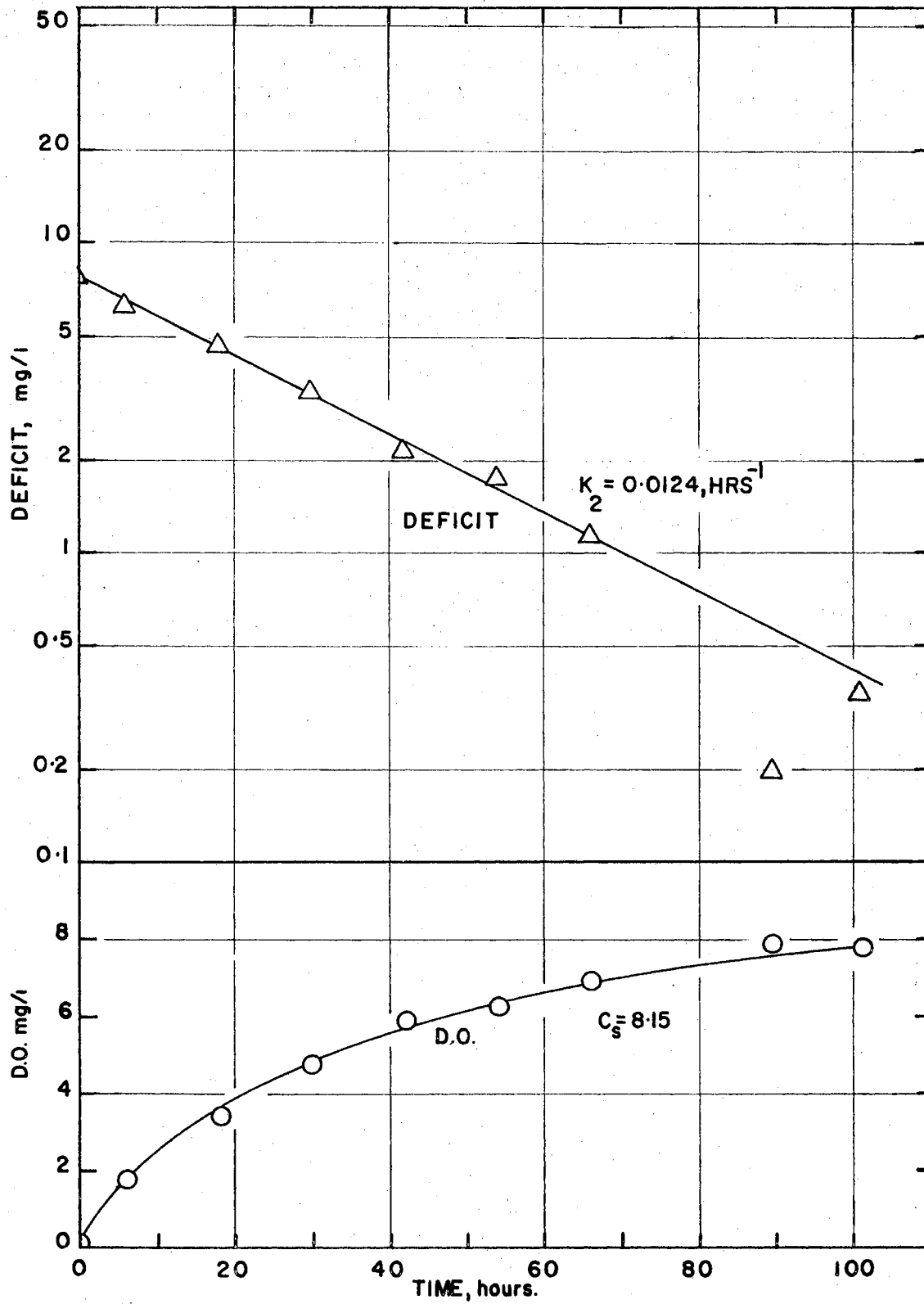


Figure 24 - Reaeration characteristics of Pond C.

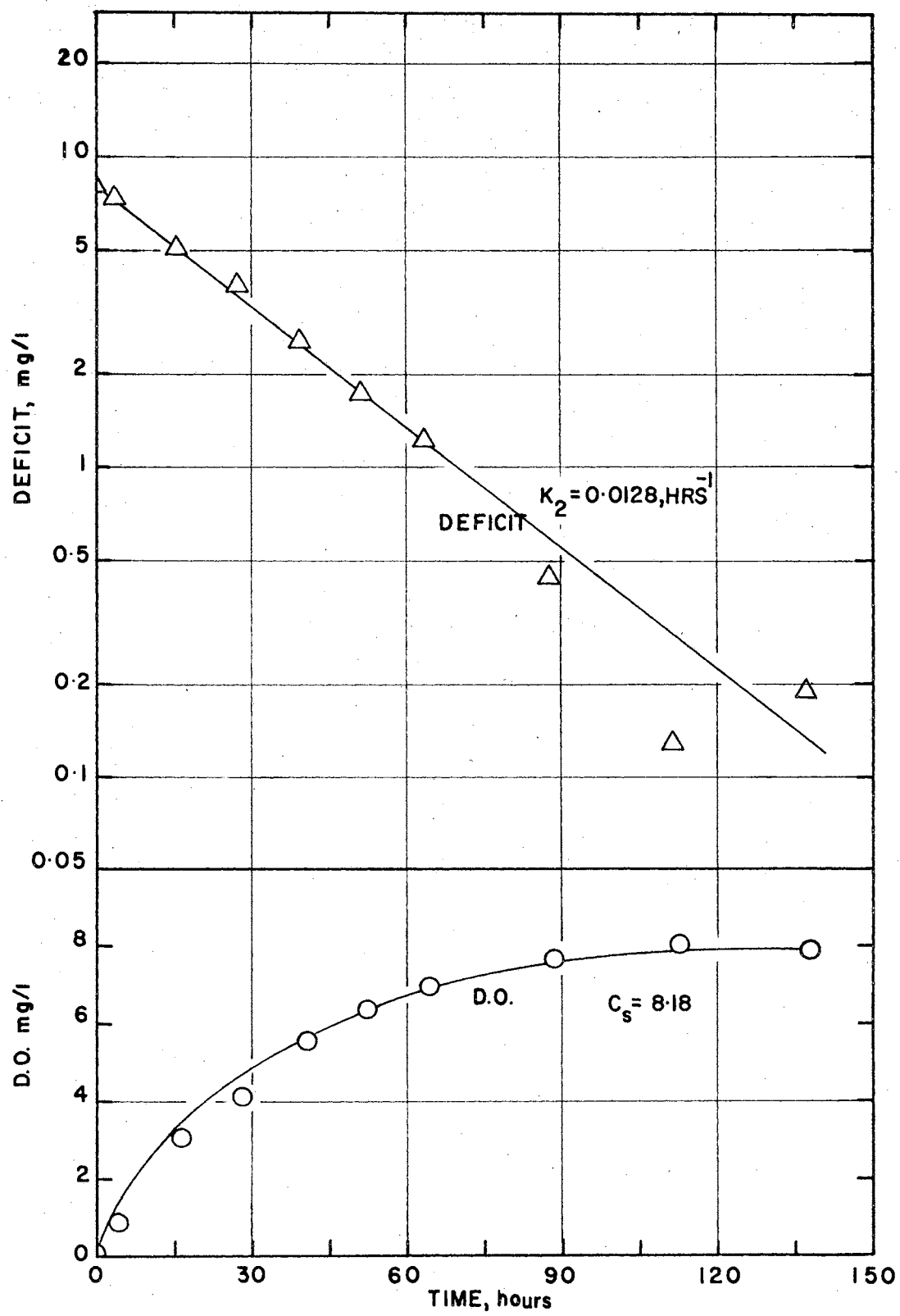


Figure 25 - Reaeration characteristics of Pond D.

systems to show the oxygen uptake curve. For the calculation of the uptake rate, the oxygen uptakes were plotted on semilogarithmic paper. The points for this plot were taken from the arithmetic plot of oxygen uptake. The slope of the line on semilogarithmic paper during the logarithmic growth phase (increasing first order rate) was calculated and designated as  $K_1$ .

Figure 26 shows the results of an experiment with 40 mg/l glucose. The  $K_1$  values for the pond were  $0.104 \text{ hr}^{-1}$ , and for the BOD bottles,  $0.082 \text{ hr}^{-1}$ .

Figure 27 shows the deoxygenation pattern in the pond for a duplicate run with the same substrate concentration (40 mg/l). The value of  $K_1$  for the pond for this experiment was  $0.113 \text{ hr}^{-1}$ .

Figures 28 and 29 show the results of experiments with a substrate loading of 60 mg/l. The  $K_1$  values (in  $\text{hr}^{-1}$ ) for the pond were 0.128 and 0.117, and for the BOD bottles they were 0.134 and 0.076, respectively. Figures 30 and 31 show the response of the systems to a loading of 80 mg/l. The deoxygenation constants were  $0.162$  and  $0.115 \text{ hr}^{-1}$  for the pond, and  $0.116$  and  $0.102 \text{ hr}^{-1}$  for the BOD bottles.

Figures 32 and 33 show the course of oxygen removal for a loading of 100 mg/l in both systems. The  $K_1$  values (in  $\text{hr}^{-1}$ ) were 0.161 and 0.135 for the pond, and 0.123 and 0.084 for the BOD bottles.

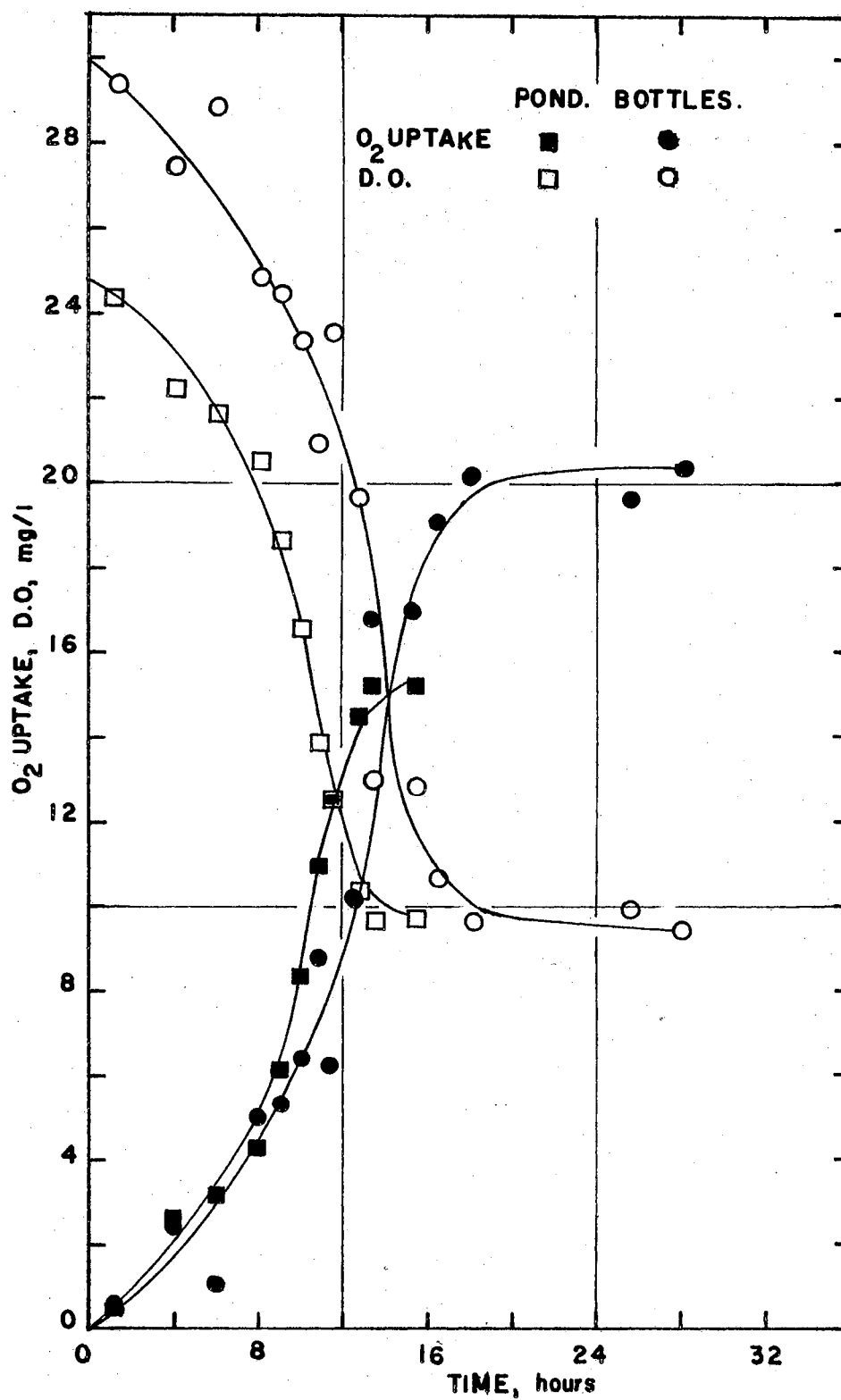


Figure 26 - Deoxygenation by organotrophs in systems with 40 mg/l glucose.

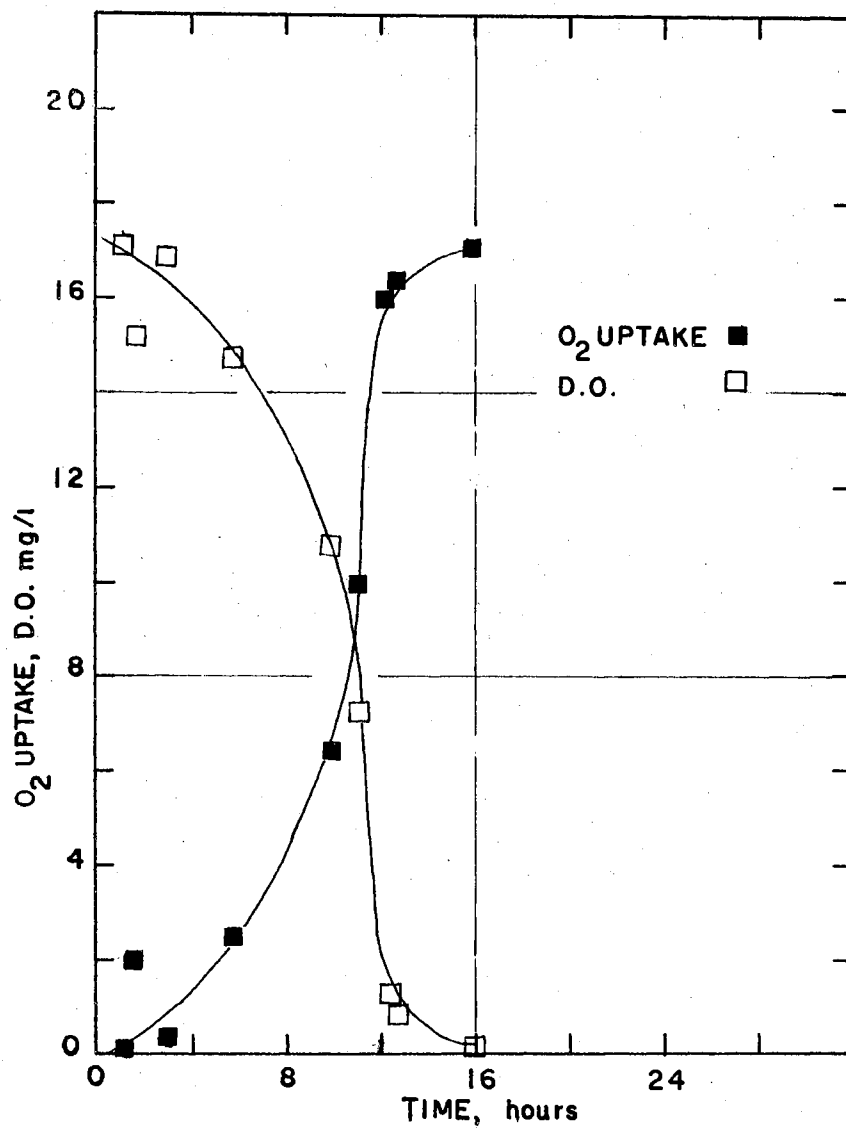


Figure 27 - Deoxygenation by organotrophs in pond with 40 mg/l glucose (duplicate run).

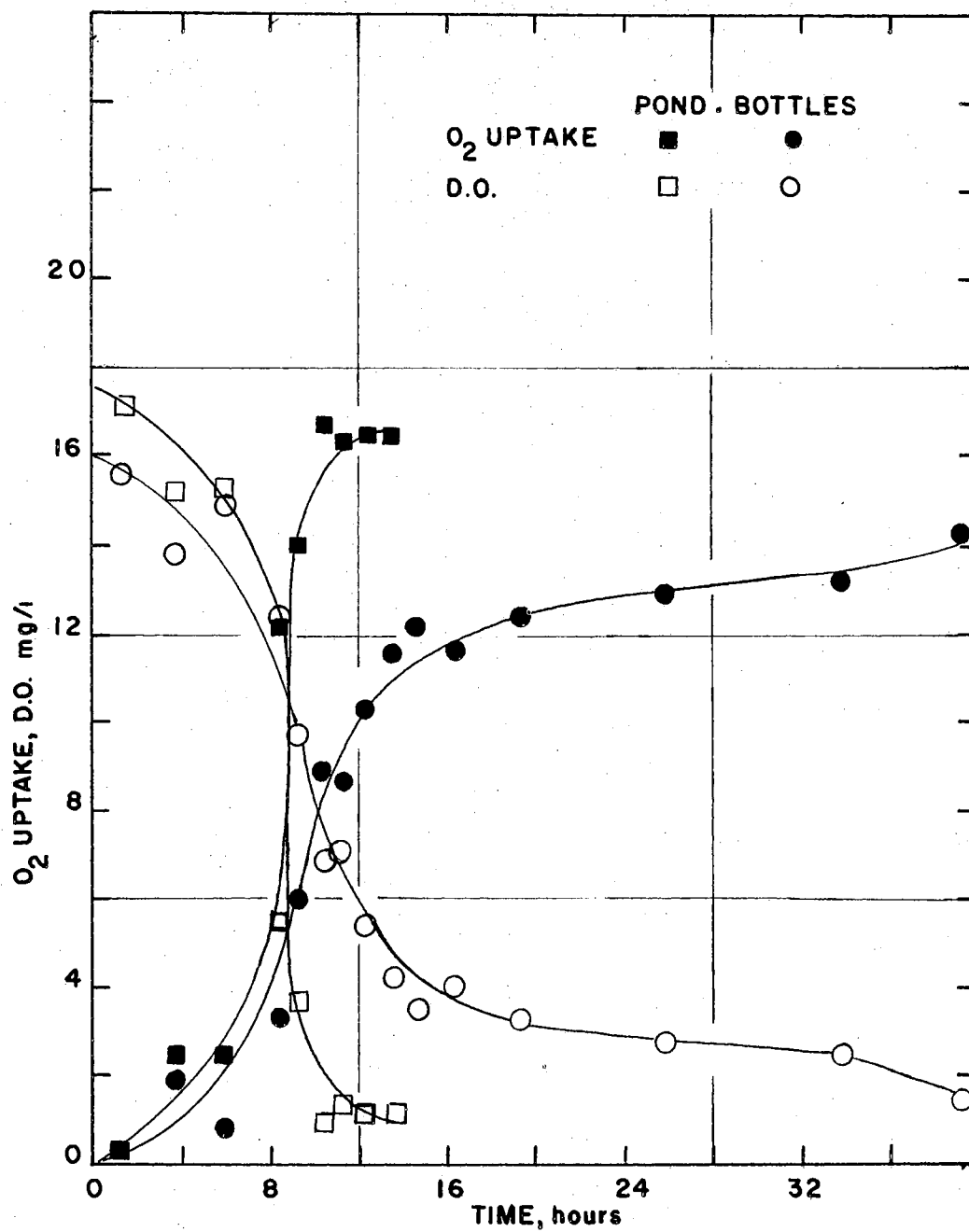


Figure 28 - Deoxygenation by organotrophs in systems with 60 mg/l glucose.

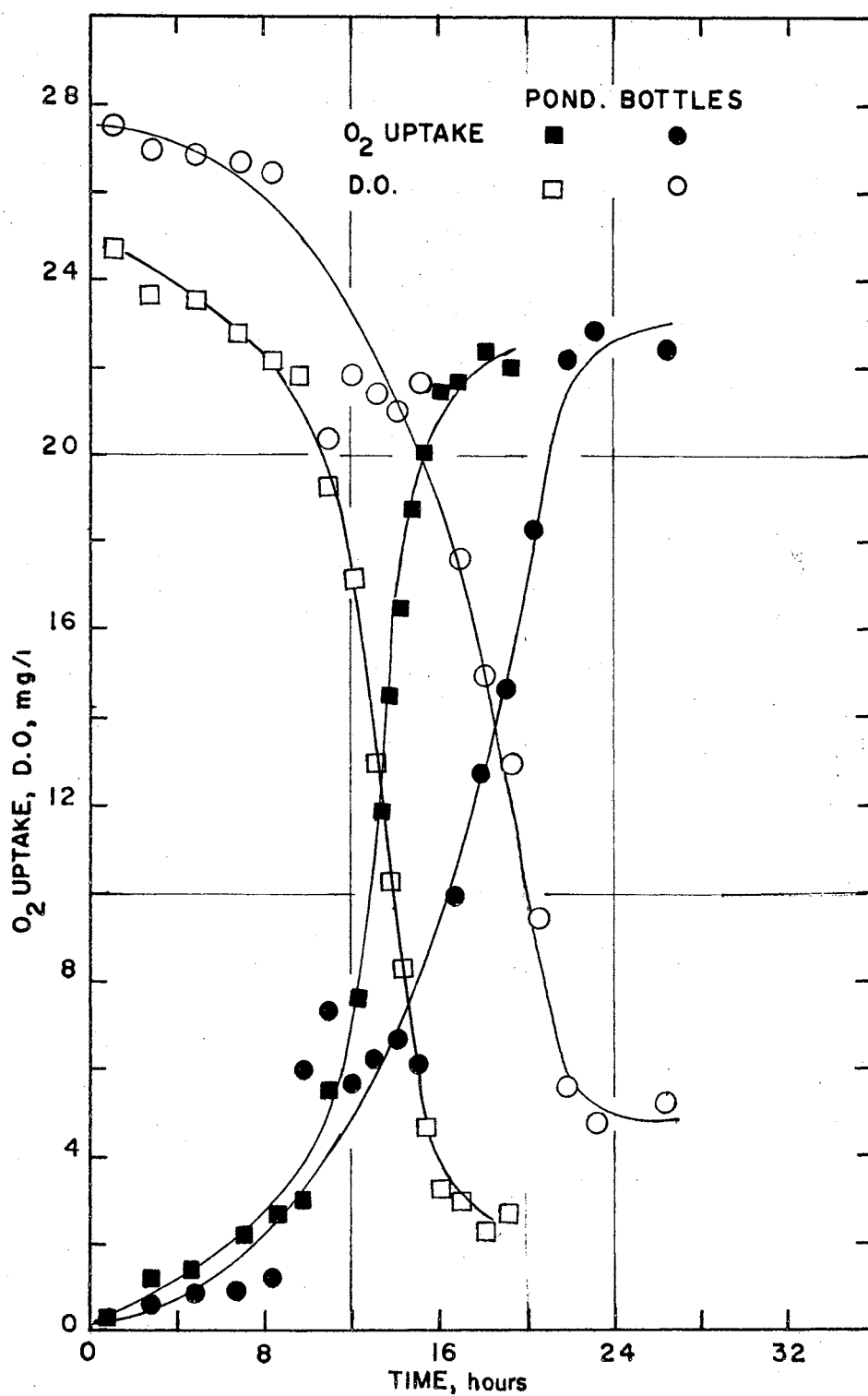


Figure 29 - Deoxygenation by organotrophs in systems with 60 mg/l glucose (duplicate run).

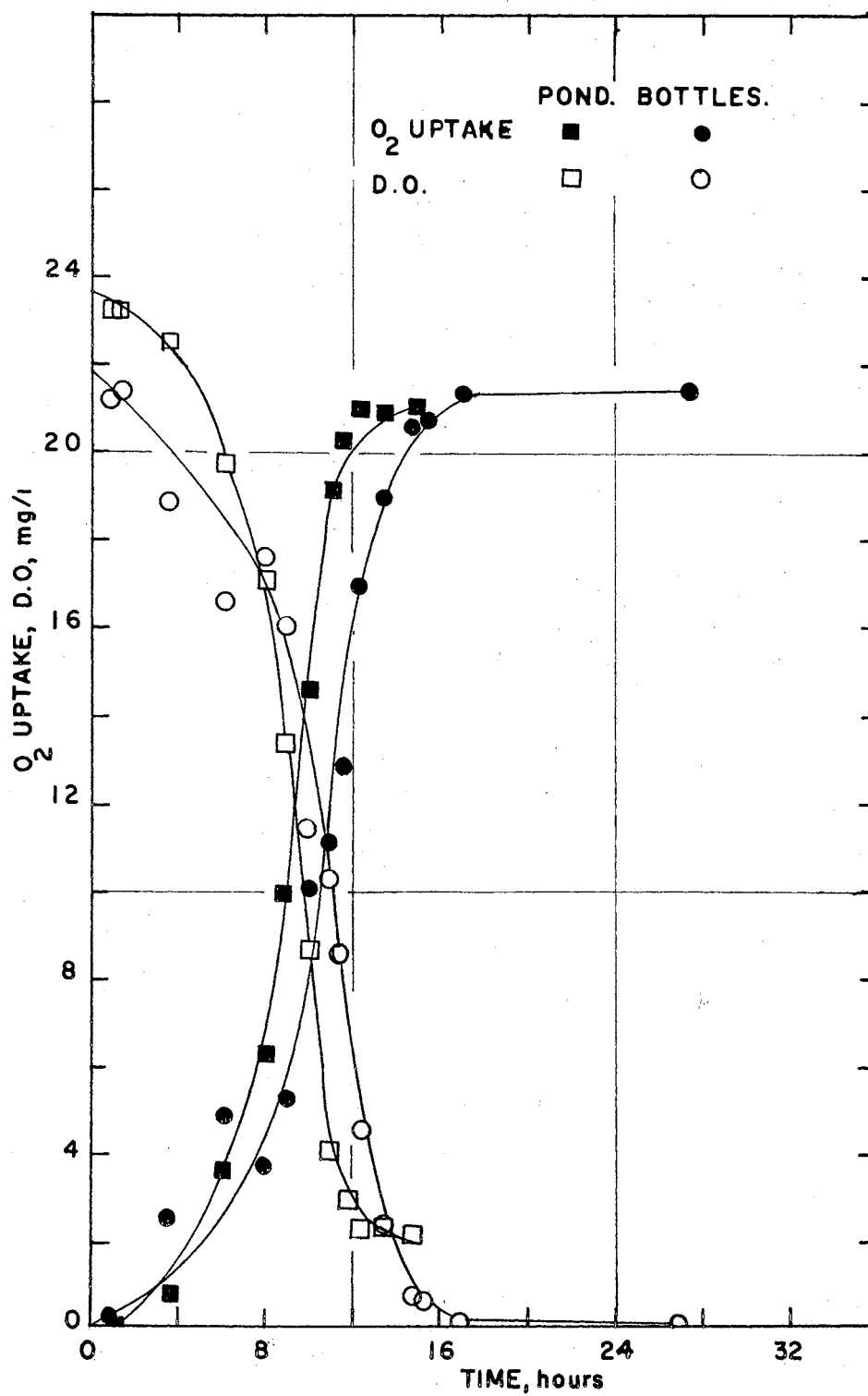


Figure 30 - Deoxygenation by organotrophs in systems with 80 mg/l glucose.



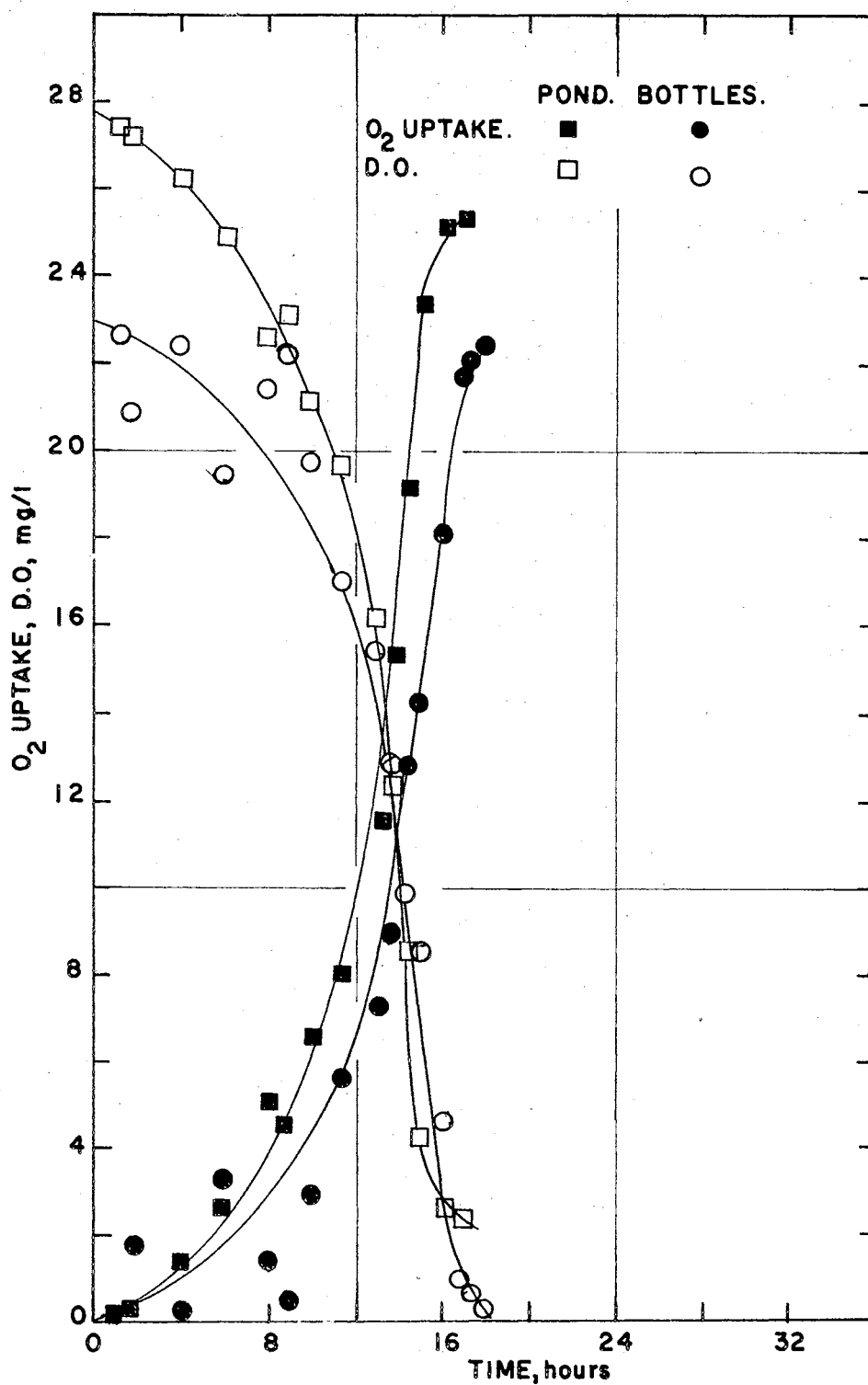


Figure 31 - Deoxygenation by organotrophs in systems with 80 mg/l glucose (duplicate run).

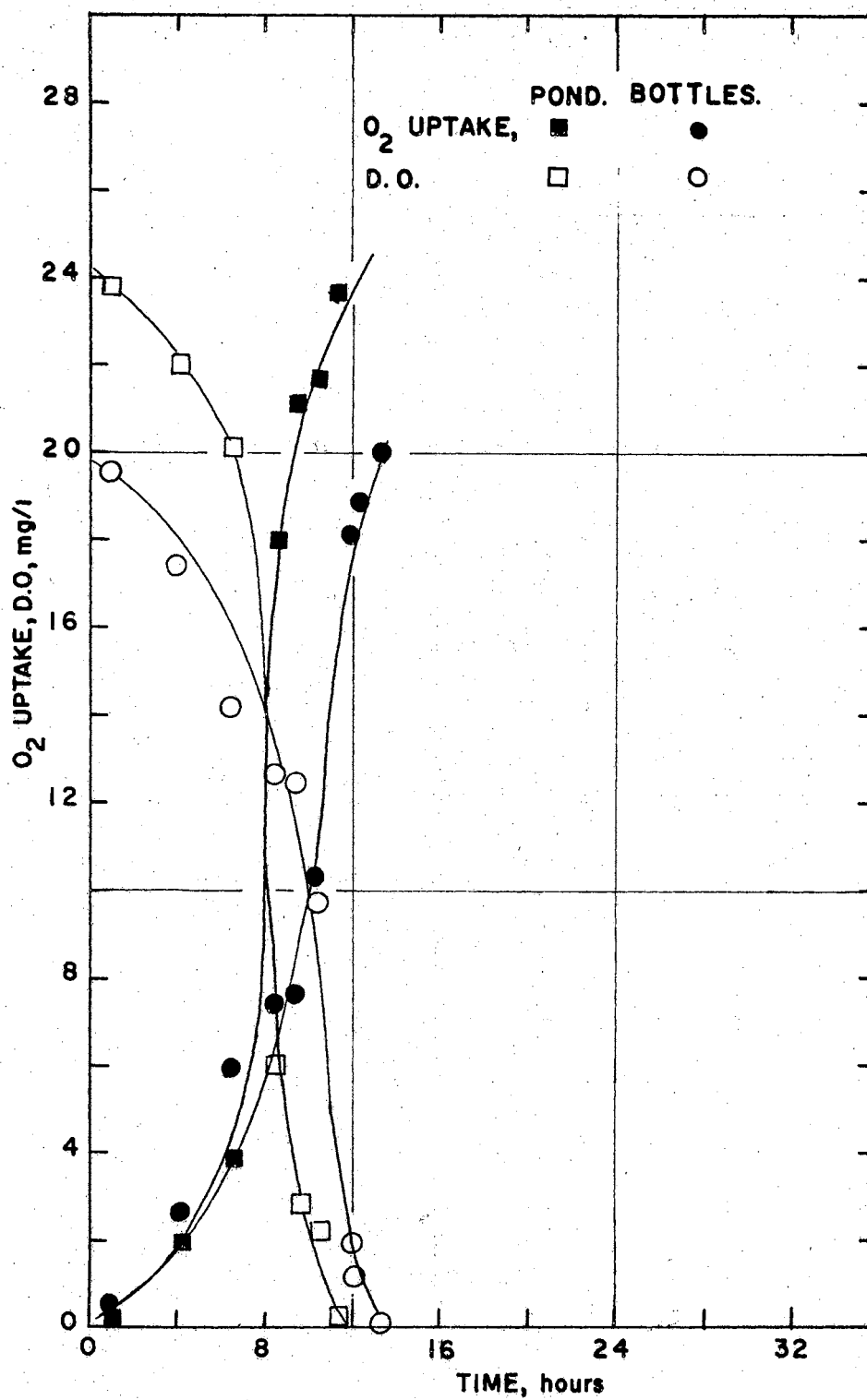


Figure 32 - Deoxygenation by organotrophs in systems with 100 mg/l glucose.

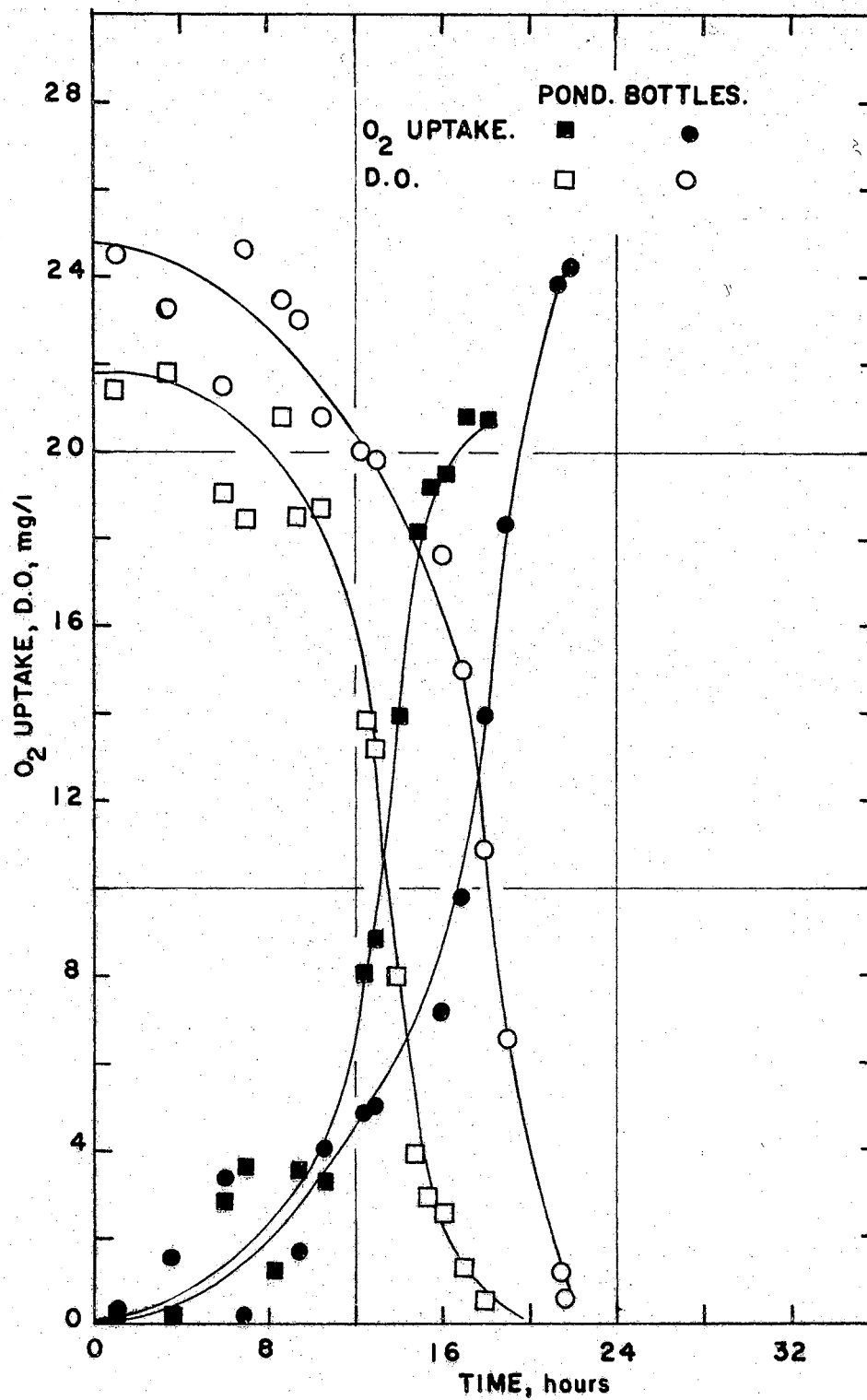


Figure 33 - Deoxygenation by organotrophs in systems with 100 mg/l glucose (duplicate run).

## B. Oxygen Utilization by Photo-autotrophs (*Chlorella pyrenoidosa*)

### Heterotrophic Metabolism

Figures 34 to 38 show the oxygen utilization and other related parameters for *Chlorella pyrenoidosa* in batch systems (Warburg flasks) containing an initial concentration of 500 mg/l glucose with various initial solids concentrations. Figure 34 shows the response of a system with an initial algal concentration of 896 mg/l. COD removal was accomplished within seven hours. The COD increased slightly for the next thirty-five hours, and then decreased slightly during the last six hours of the experiment. The solids increased considerably during the first seven hours. After reaching a maximum at nine hours, the biological solids concentration decreased a very small amount during the period in which the COD showed a slight increase.

The oxygen uptake curve followed zero order kinetics during active substrate removal, and then dropped off at a decreasing rate; however, during the last six hours the curve turned upward. In view of the decrease in both COD and solids during this period, it seems that the second stage of oxygen utilization might have proceeded due, partially, to secondary growth using the lysed products of the cells. The carbohydrate curve shows that metabolic intermediates and/or end products produced during active substrate removal were in very small amount, and that the residual COD was not due to carbohydrate.

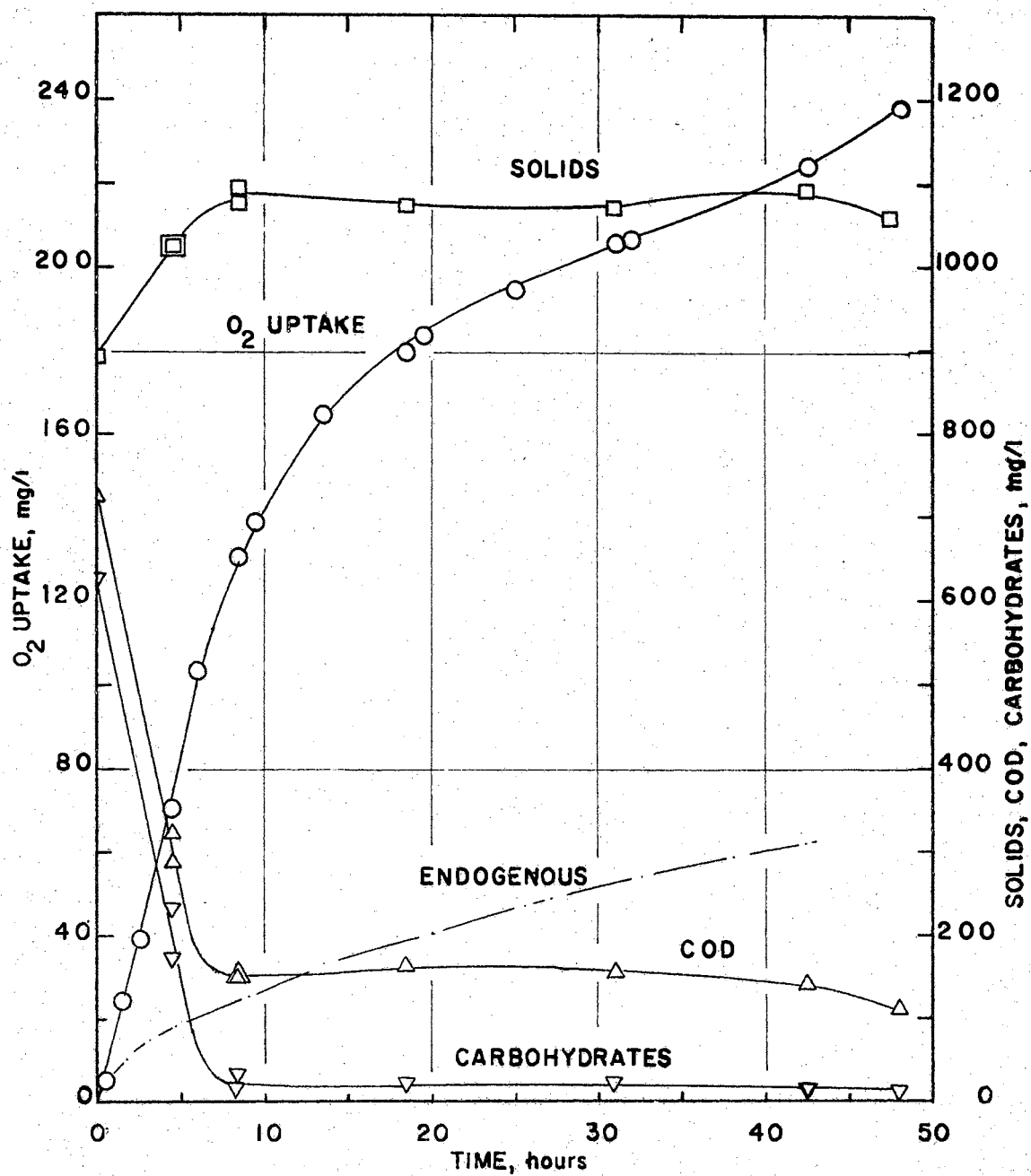


Figure 34 - Deoxygenation by photo-autotrophs;  
initial algal concentration 896 mg/l.

Figure 35 shows the response of a system with an initial algal concentration of 448 mg/l. The general pattern is the same as that for the previous system. The COD was removed in approximately eleven hours. The carbohydrate curve paralleled the COD curve, indicating no accumulation of metabolic products. The biological solids increased during the period of substrate removal, and then leveled off. The oxygen uptake curve indicated a slightly increasing rate for eleven hours, and a decreasing rate thereafter.

Figure 36 shows the response of a system with an initial algal concentration of 400 mg/l. The performance was very similar to the system described in Figure 35. The increasing trend in the oxygen uptake rate was more pronounced during the period of active substrate removal, and after removal of the COD the rate was linear.

Figure 37 shows the response of a system with an initial algal concentration of 215 mg/l. The active substrate removal period was twelve and one-half hours. There were no metabolic intermediates or end products during this active substrate removal, as indicated by comparison of carbohydrate and COD curves. The small residual COD and carryover COD were not due to carbohydrates. The biological solids concentration increased until the end of the COD removal period. Immediately after attainment of the maximum value, the solids concentration decreased slightly and thereafter rose slightly. The oxygen uptake curve indicates that a logarithmic growth phase was attained. After

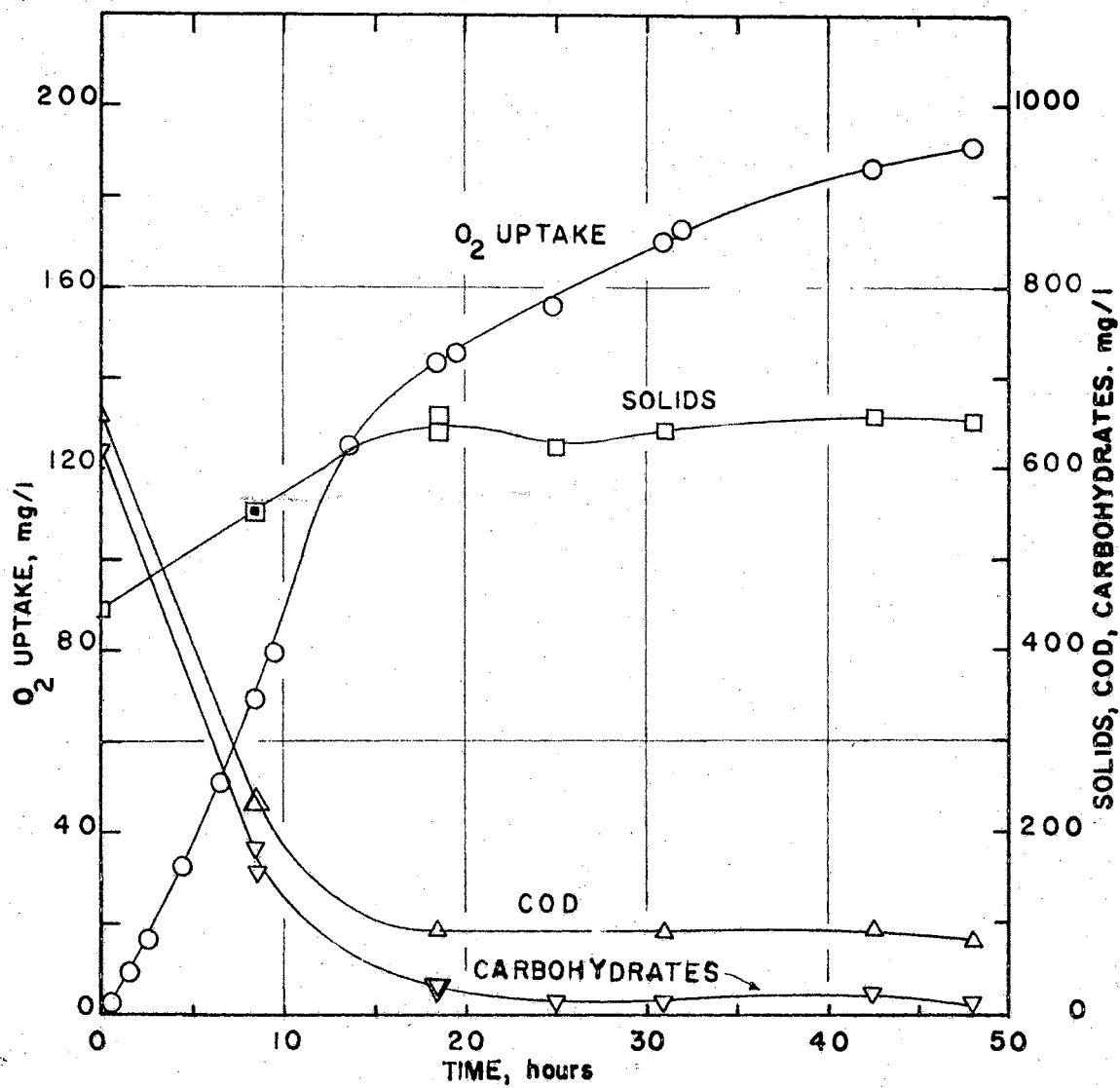


Figure 35 - Deoxygenation by photo-autotrophs; initial algal concentration 448 mg/l.

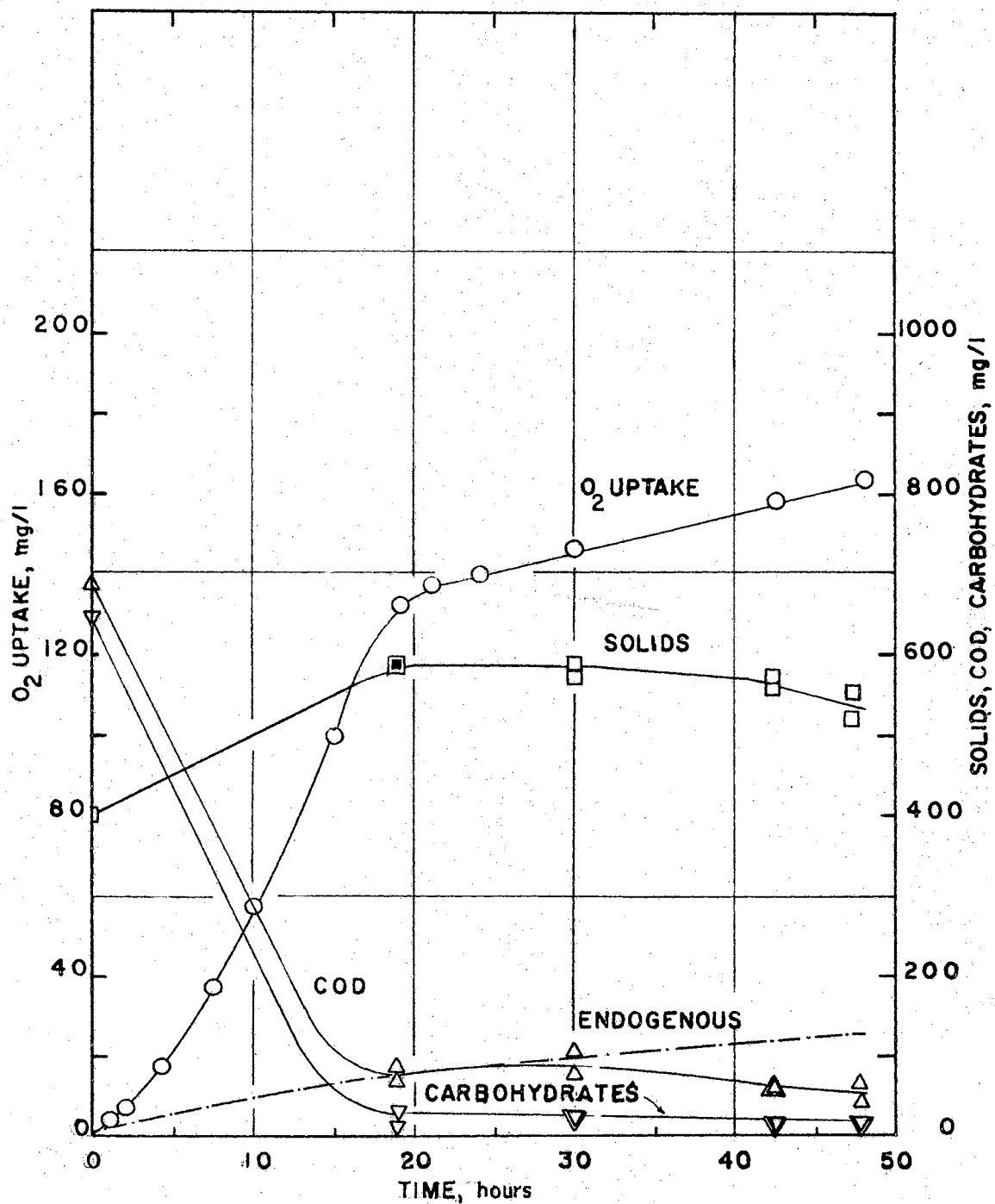


Figure 36 - Deoxygenation by photo-autotrophs; initial algal concentration 400 mg/l.



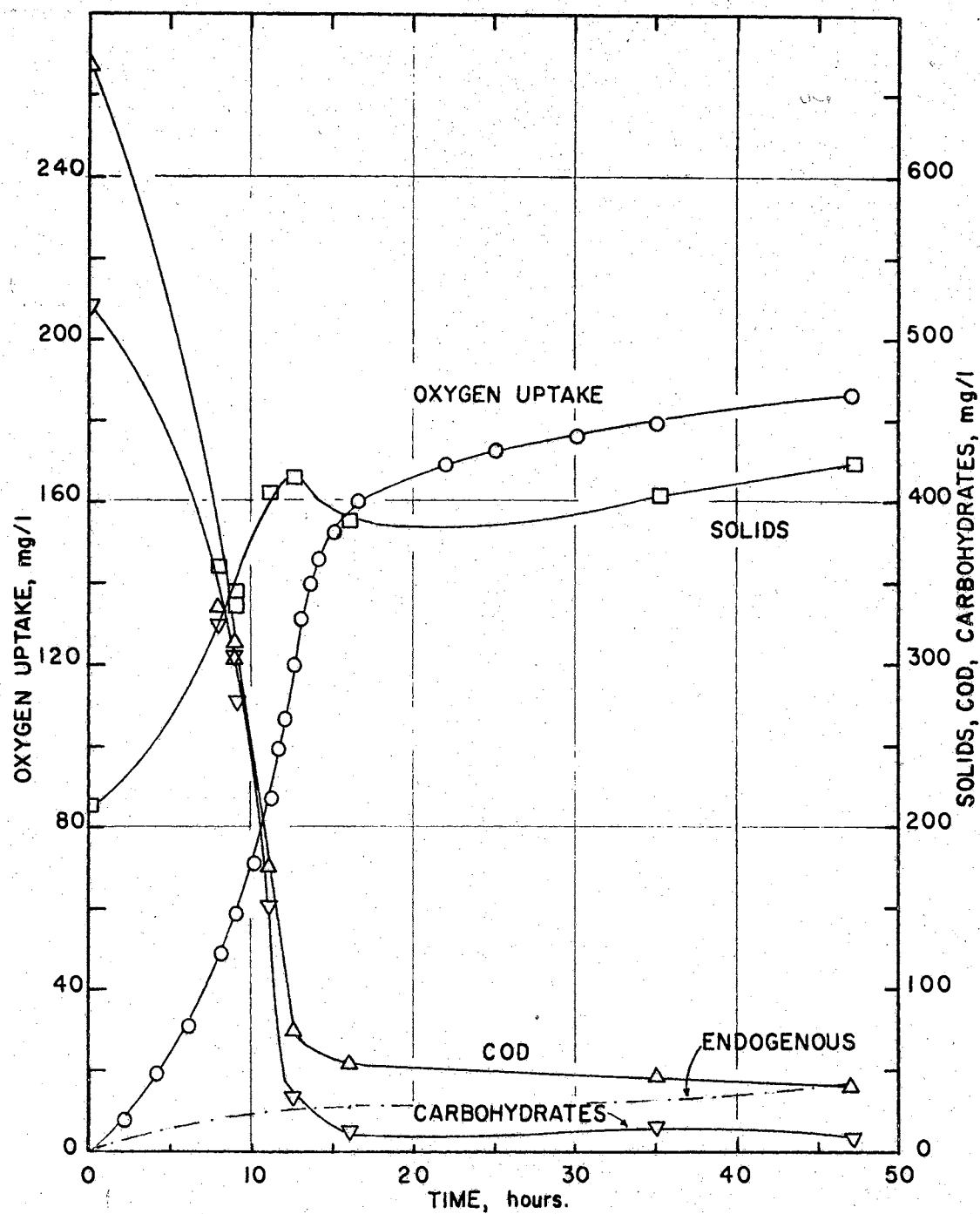


Figure 37 - Deoxygenation by photo-autotrophs; initial algal concentration 215 mg/l.

twenty-four hours the  $O_2$  uptake rate was more or less constant.

Figure 38 shows the response of a system with an initial algal concentration of 113 mg/l. The behavior was very similar to the system shown in Figure 37. The period of substrate removal and algal growth were prolonged to fifteen hours, compared to twelve and one-half hours in the previous system.

#### Endogenous Metabolism

Figures 39, 40, and 41 show the oxygen uptake and other related parameters for Chlorella pyrenoidosa during endogenous metabolism with various initial solid concentrations. Figure 39 shows the endogenous oxygen uptake, solids concentration and filtrate COD with respect to time for a system with an initial solids concentration of 1325 mg/l. Eight Warburg flasks were used in the beginning of the run. After 24, 48, and 96 hours, duplicate flasks were removed, and one of each was removed at 72 and at 120 hours for solids and COD determinations. The arrows on the oxygen uptake curve show the time of removal of flasks. The biological solids concentration gradually decreased from 1325 to 1164 mg/l, a decrease of 161 mg/l. The filtrate COD increased by approximately 20 mg/l.

The oxygen uptake in all flasks was uniform. For example, after an elapsed time of seventy hours, the cumulative oxygen uptakes in the four remaining flasks were 126, 120, 118, and 117 mg/l. The cumulative uptake in the

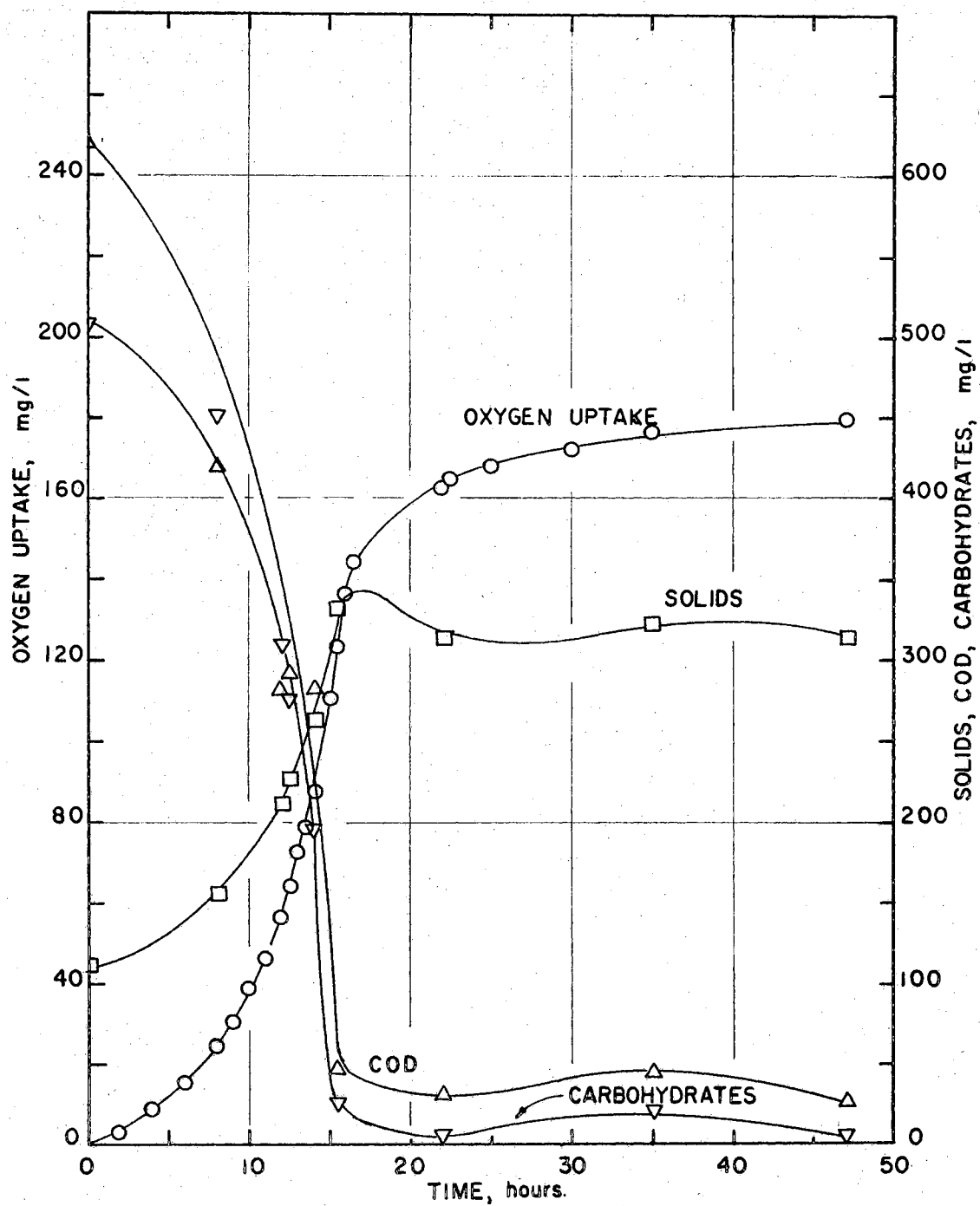


Figure 38 - Deoxygenation by photo-autotrophs; initial algal concentration 113 mg/l.

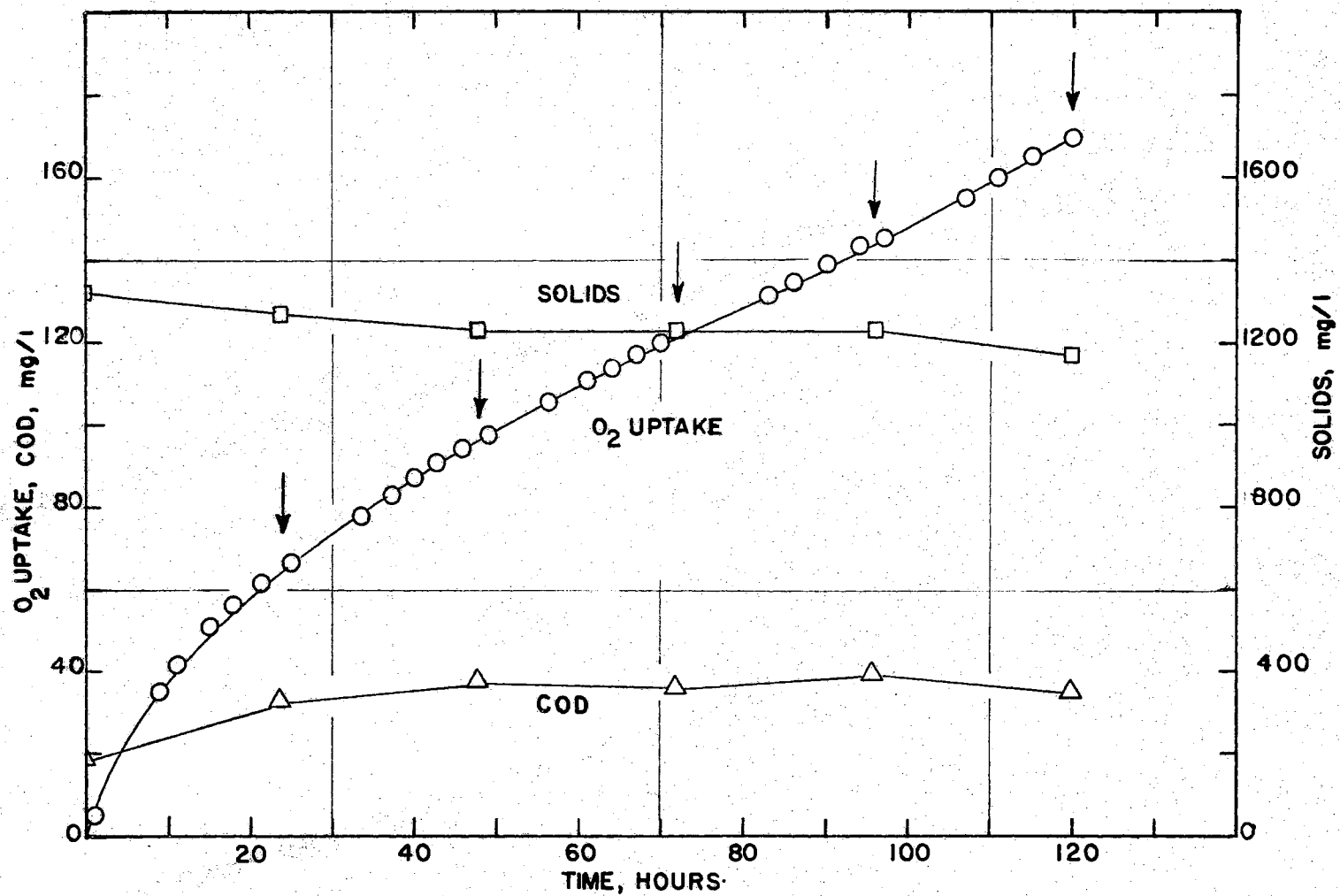


Figure 39 - Endogenous metabolism of *Chlorella pyrenoidosa*; initial algal concentration 1325 mg/l.

flask that remained at 120 hours was 170 mg/l. The oxygen uptake curve appears to follow a first order decreasing rate until eighty hours. Using the  $\alpha$  method described in detail elsewhere (81), the ultimate demand was calculated by selecting three points within the 80-hour range. On this basis the ultimate demand was 195 mg/l, and the rate constant was  $0.0056 \text{ hr}^{-1}$ . As a verification of the kinetic mode of the oxygen uptake curve, the observed  $\text{O}_2$  uptake values at various times were deducted from the ultimate demand of 195 mg/l, and were plotted on semi-logarithmic paper. The points, up to ninety hours, did fall on a straight line. The calculated slope of that line, the rate constant, was  $0.0054 \text{ hr}^{-1}$ .

Figure 40 shows the endogenous oxygen uptake for systems with final algal concentrations of 532, 316, and 220 mg/l. Five Warburg flasks were set up for each system. One flask in each system was removed at 60, 88, 120, 144, and 168 hours for determination of solids and filtrate COD. The oxygen uptakes were uniform in all flasks belonging to the same group. For the system with final solids of 532 mg/l, after 88 hours the cumulative oxygen uptake values for the remaining flasks were 73, 71, 75, and 74 mg/l. The cumulative uptakes in one week's time (168 hours) were 116, 45, and 21 mg/l, respectively, for the three systems. Here, also, the first portion of the curves follows a first order decreasing trend. Using the same method of calculation as in the previous case, the rate constants were calculated.

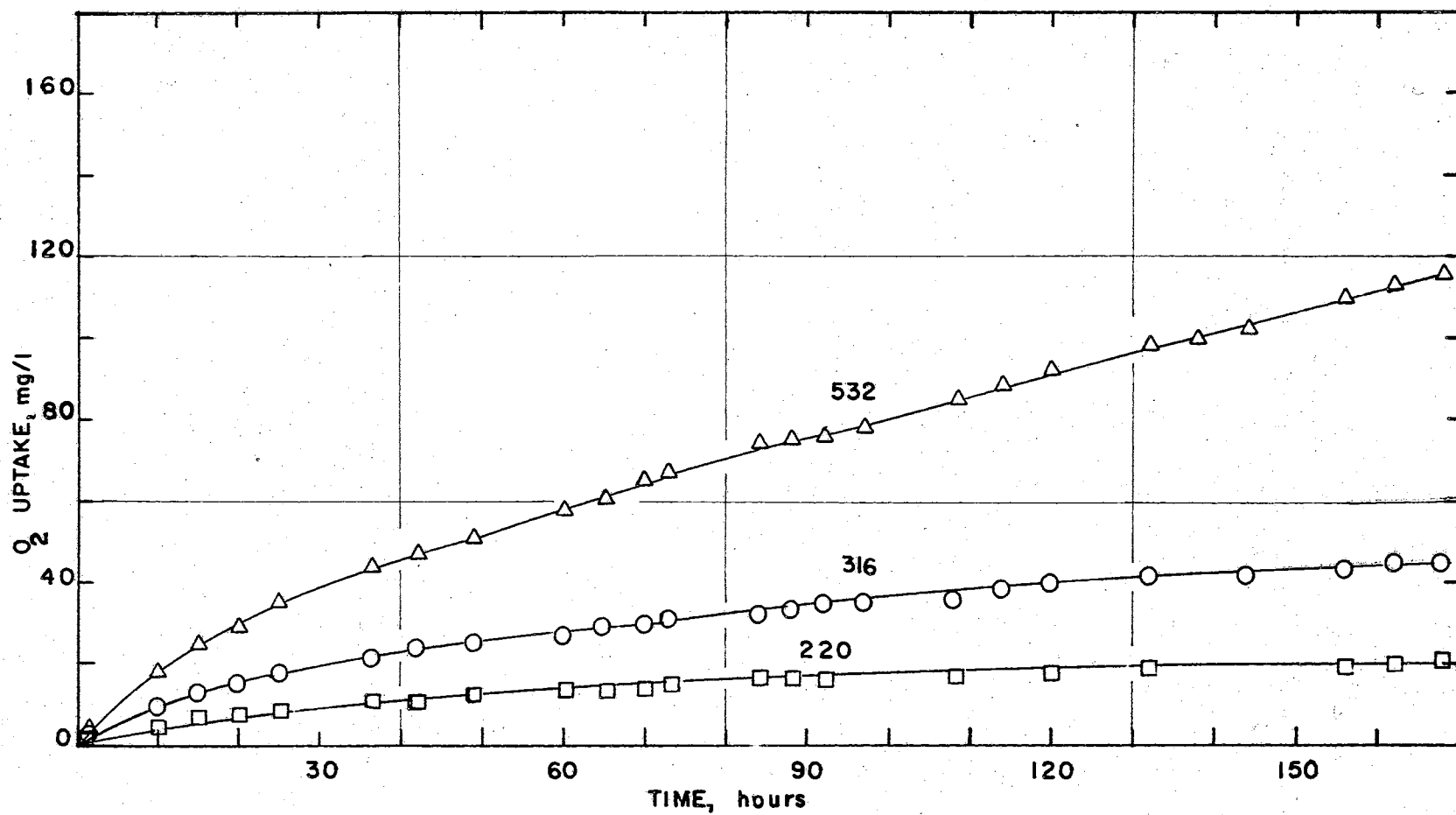


Figure 40 - Endogenous oxygen uptakes for indicated algal concentrations.

They were 0.0051, 0.0061, and 0.0063  $\text{hr}^{-1}$ , respectively.

Figure 41 shows the biological solids and filtrate COD values for the three systems. The changes in solids and COD concentrations were very small. However, the general pattern of solids shows a slight reduction for the 532 mg/l system, no change in the 316 mg/l system, and a slight increase in solids concentration for the 220 mg/l system. The filtrate COD increased slightly for the high solids system, whereas in the other two there was a small decrease in filtrate COD during the course of endogenous respiration.

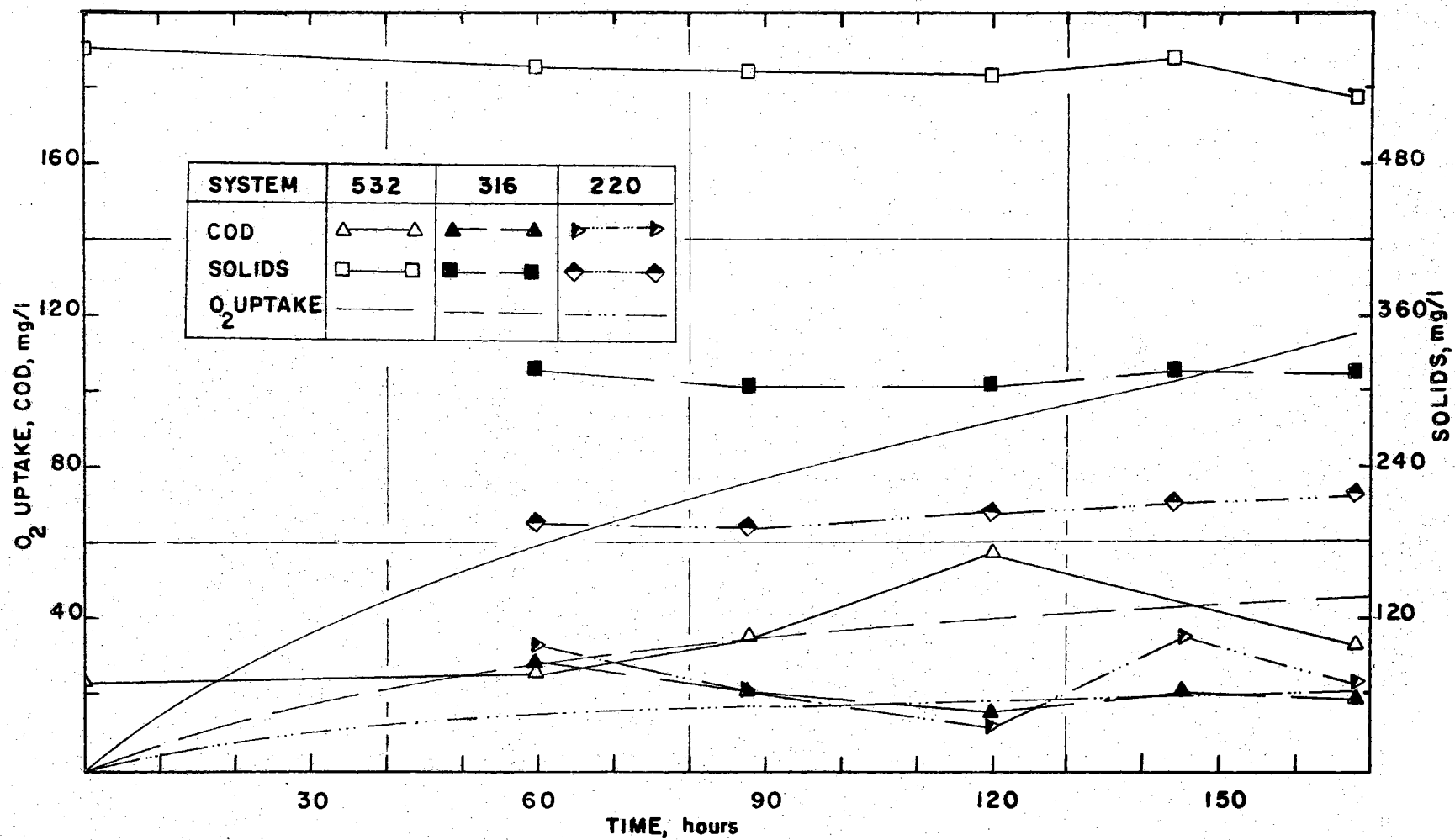


Figure 41 - Endogenous metabolism of *Chlorella pyrenoidosa* in systems with indicated algal concentrations.



## CHAPTER VI

### ANALYSIS OF RESULTS AND DISCUSSION

The effect of free gas transfer across the liquid surface interface of the pond on the performance of the experimental units can be evaluated by comparison of substrate removal efficiencies at the same loading levels when the free transfer was unobstructed and when it was totally prevented. For such a comparison, results of the experiments with feed concentrations of 100, 150, and 250 mg/l glucose in open and closed systems are considered.

The response of the open system to a loading of 100 mg/l glucose (Figure 7) showed two patterns. The dissolved oxygen levels at the end of light and dark periods, and the changes in DO were uniform during the first ten days of operation. Even though these parameters were also uniform in the last five days of operation, after the fifteenth day their values were quite different from those of the first ten days of operation. For example, the average DO change in the first ten days was approximately 7.5 mg/l, compared to 4.5 mg/l in the last five days. The biological solids and the oxidation reduction potential showed a steady decline from the eleventh day onward, and both reached minimum values at the end of the fourteenth day. The

decrease in solids might have been due to the buildup of autotoxic material (31)(48)(62)(63), or due to the constituents brought in by the tap water (71). Since there was a change in the color of the mixed liquor (darker to lighter green) there is some indication that both algal and bacterial solids were decreased. The decreased oxygen production in the last five days also indicated the possibility of a decrease in algal concentration. The increase in the biological solids after the fifteenth day might have been due mainly to bacteria, as the COD was reduced considerably during the period corresponding to the solids increase and since there was neither an increase in dissolved oxygen production nor an appreciable change in the intensity of the green color of the mixed liquor. The first ten days of operation were taken as the period of most "balanced operation," and the average for various parameters in that period are given in Table III.

In the open pond which received 150 mg/l glucose (Figure 8), all parameters were more or less steady except ORP. The ORP increased as the run progressed, indicating a decrease in the reductants and an increase in the oxidized materials (e.g., nitrate, although such analysis was not run). The last seven days of the run were taken as the most representative period of uniform operation, and the averages of those parameters are given in Table III. The open pond subjected to a loading of 250 mg/l glucose (Figure 9) behaved satisfactorily for five days, but after

TABLE III  
AVERAGE VALUES OF PARAMETERS DURING BALANCED OPERATION  
IN CONTINUOUS FLOW PONDS

Parameters Averaged	Open System			Closed System					
	100	150	250	100			150	250	
				14th to 23rd	24th to 36th	14th to 36th		0-9 days	Regenerated
Figures, #	7	8	9	11	12	11,12	14	15	17
Maximum DO, mg/l	9.6	4.7	10.4	33.1	22.6	27.1	30.1	34.4	13.9
Minimum DO "	2.1	0.4	0	18.0	12.8	15.0	11.0	10.9	0.3
Daily Change "	7.5	4.3	10.4	15.1	9.8	12.1	14.1	23.5	13.5
Biological Solids "	91.9	91.5	50.0	57.0	50.0	53.0	59.0	73.0	73.0
Effluent COD "	114.1	90.5	64.5	48.0	45.0	46.6	65.0	40.0	56.0
COD Removal %	0	46.0	76.0	58.0	60.5	59.0	61.0	86.0	80.0

ten days the system failed with regard to oxygen production. The DO increase was more or less uniform in the first five days, and in the second five days it decreased progressively until it reached zero. The biological solids concentration was greater, whereas the pH level was lower in the second five days compared to the first five days of operation. The increase in solids in the latter half of the run was probably due to bacterial growth, since there was no corresponding DO increase in the light period, as would be expected from a greater algal population. This increase in bacterial cells could be expected to cause greater turbidity which, in turn, would further reduce the algal growth and oxygen production, finally bringing the DO level to zero. The reduced algal activity could have resulted in the slightly lower pH level in the second half of the operational period. It is suggested that the sequence of events described above is typical of the progressive deterioration of efficiency in oxidation ponds approaching overloaded conditions. The data of the first five days were taken as being representative of "balanced operation," and the average values are compared to those for the other systems in Table III.

The average DO change in the first nine days of operation for the closed pond at a loading of 100 mg/l glucose (Figures 10, 11, 12) was lower in comparison with the daily DO changes observed thereafter. Since there was no increase in the total biological solids concentration (in fact,

there was a slight reduction) during the latter part of the run, it is possible that the biomass might have consisted of more bacteria than algae in the initial stages. The capacity of bacteria to grow faster than the algae (e.g.,  $\mu = 0.18 - 0.15 \text{ hr}^{-1}$  for bacteria vs.  $0.09 \text{ hr}^{-1}$  for algae) might have caused this. As pointed out in the previous chapter, there were two periods of "balanced operation:" one for nine days, and the other for twelve days. The average values for a 22-day period consisting of these two "balanced operation" periods are given in Table III. The smaller changes in daily DO levels at the beginning of the run were also observed in the closed pond with the influent concentration of 150 mg/l (Figures 13, 14). "Balanced operation" was attained after ten days of operation until the end of the run. The behavior of the system on the thirteenth and fourteenth days was somewhat unique. During the light period of the thirteenth day the DO increase was small, almost half, compared to the increase for both the preceding and following days. This resulted in a lower DO level at the end of the succeeding dark period. The ORP also dropped considerably during the light period on the thirteenth day. For some (unknown) reason the system was thrown out of balance temporarily on that day. Possibly the algal metabolism might have been retarded on that particular day by certain metabolic products in the pond. The algal activity, indicated by the increase in DO during the light period, on the next (fourteenth) day was the maximum

attained - 18.2 mg/l against an average of 14.1 mg/l - during the period of "balanced operation." This was further reflected by a sharp increase in pH during the same period.

The closed pond which received 250 mg/l of glucose (System I, Figures 15 and 16) operated under "balanced conditions" for the first nine days. The dark green color of the reactor liquor became yellowish-brown after nine days, and the pond began to fail with respect to maintenance of oxygen level. The highest DO concentration, measured in this series of investigations, 38 mg/l, was recorded in this system, and the COD removal efficiency was 86 per cent. The observed decrease in DO levels and the subsequent failure of the system during the second half of the run was due to the reduced production of oxygen during the light rather than to any increased utilization of  $O_2$  in the dark. The "regenerated" system, which received the same load, 250 mg/l glucose (Figure 17) was light green in color and responded more or less in a uniform manner throughout the period of observation. The DO levels at the end of the dark period were close to zero (on two days zero readings were recorded) which indicated that the system was under maximum possible load for maintenance of aerobic conditions. The sharp decrease in ORP for short durations and the subsequent recovery to the original level may also be taken as an indication that the system was delicately balanced with respect to maintenance of aerobic conditions at this loading level. Average values of the operational parameters of

this system are given in Table III. A closed pond operated at an equivalent loading factor (twice the detention time and double the organic loading -- twenty days detention and 500 mg/l glucose) was completely anaerobic (Figure 18). The COD removal decreased from 88 per cent to 77 per cent during the period of observation. The maximum ORP was -50 millivolts at the end of the light period on the second day.

Comparison of parameters (Table III) for the experiments on the open system (Figures 7, 8, 9) would enable assessment of the effect of organic loading on the performance of the ponds. As the influent load was increased from 100 to 150 and 250 mg/l glucose in the three open pond systems, the intensity of the green color decreased. The biological solids level decreased surprisingly; values of 91.9, 91.5, and 50.0 mg/l, respectively, were recorded in the three systems. However, the decrease in solids level did not lead to a decrease in COD removal. The COD of the effluent filtrates were 114, 91, and 65 mg/l, corresponding to COD removals of 0, 46, and 76 per cent. It seems quite obvious from these results that at the lower organic loading more carbon was fixed than was removed. Whereas the carbon balances in these systems cannot be made, the two measured parameters, effluent COD, and biological solids, may be employed to obtain a rough idea of the sources and sinks of carbon.

Although the elemental compositions for different species are different and vary even within the species,

depending on growth environment, an average figure of 51 per cent of the weight of the biological solids may be attributed to carbon (31)(90). Since the value of the influent COD and the nature of the substrate were known, the influent organic carbon can be estimated fairly accurately by multiplying the average influent COD values by a factor 12/32 (192 mg of oxygen is required to oxidize 72 mg of carbon in glucose). However, determination of the organic carbon of the effluent may not be done accurately, because of the uncertainty of the nature of the effluent. Spot checks for glucose by the Glucostat test showed the absence of glucose. As seen in the literature review (55)(56)(57)(59)(60), the major excretory products of algae have been reported to be polysaccharides and organic acids of low molecular weights. Studies in this laboratory (91)(92)(93) showed that acetic acid was a major constituent in the intermediate products when heterogeneous bacteria metabolized glucose. Taking into account all of these points, a multiplication factor of 12/32 may be used for the estimation of effluent COD. (It might be noted that the carbon equivalent of acetic acid COD is also 12/32) Furthermore, since these calculations are for a comparison between systems under various loadings (for all of which the same method is adopted), any possible difference in the true value of organic carbon and the calculated values will apply to all systems and will



not significantly affect the comparison. On this basis the carbon contents in the solids of the three systems were 46.9, 46.6, and 25.5 mg/l. The carbon equivalents of the effluent COD were 42.8, 33.9, and 24.2 mg/l for systems with the influent loading of 100, 150, and 250 mg/l glucose, respectively. Addition of the carbon equivalents of effluent COD and solids yield 89.7, 80.5, and 49.7 mg/l output against an input of 42.8, 63, and 102 mg/l. These figures indicate a carbon addition of 46.9 and 17.5 mg/l in systems loaded with 100 and 150 mg/l glucose, and a carbon decrease of 52.3 mg/l in the system receiving glucose at 250 mg/l. The organic carbon addition could arise by  $\text{CO}_2$  removed from the carbonate system of the medium and by the fixation of  $\text{CO}_2$  from the atmosphere. The presence of 100 mg/l sodium bicarbonate in the feed could contribute carbon to a maximum amount of 14.3 mg/l ( $100 \times 12/72$ ). Under the conditions of the closed pond experiments (when the carbon exchange with the atmosphere could not take place and the only additional source other than that provided by the feed was the carbonate system), the only observed contribution from the carbonate system was 1.8 mg/l of carbon. Therefore, it may be discerned that the addition of carbon to the system at lower loadings was primarily due to the fixation of atmospheric carbon dioxide. Theoretical possibilities and indications in field observations (9) (31) (75) (76) (77) (78) to this effect were pointed out earlier. Such an effect occurs only in ponds subjected to low organic

loadings. As the complement of metabolically produced  $\text{CO}_2$  increases (as it would be expected to at higher loadings), the carbon addition effect is decreased (e.g., 150 mg/l system), and finally a carbon loss is registered (e.g., 250 mg/l system). From this discussion the evidence seems to indicate that atmospheric carbon dioxide is probably fixed by algae in the open ponds, and that this is more significant at lower loading levels, and this process decreases the overall efficiency of the ponds.

A scrutiny of the performance of the closed pond (Figures 10, 11, 12, 13, 14, 15, 16, 17) will serve to reinforce the analyses and tentative conclusions cited above. In the closed ponds the COD removal efficiencies were 59, 61, and 83 (average of 86 and 80 in two systems) percent in systems with influent loadings of 100, 150, and 250 mg/l glucose, respectively. The biological solids also exhibited an increasing trend; 53, 59, and 73 mg/l. Using the same method of calculation to account for the total carbon as in the previous section, the outgoing carbon concentrations were 44.6, 54.5, and 52.2 mg/l compared to incoming carbon of 42.8, 63, and 102 mg/l. These figures indicate that the carbon in the carbonate system was not needed for solids production except possibly at the lower loading (100 mg/l glucose). On the contrary, the reduction of organic carbon in the 150 and 250 mg/l systems (as well as in the 250 mg/l open pond system) indicate that metabolic  $\text{CO}_2$  would have increased the free  $\text{CO}_2$  in the

medium and the associated carbonates.

Considering the two systems, open and closed, which were subjected to the same organic loading, the effectiveness of closing the pond can be evaluated. In the open system with 100 mg/l glucose loading (Figure 7), the influent and effluent COD were the same, whereas 50 per cent COD removal was attained in the closed pond (Figures 11 and 12). The solids concentration in the open pond was 92 mg/l versus 53 mg/l in the closed pond. On this basis, the open system can be termed a "polluting system" rather than a treatment system, and the results augur well for the closed system. At a loading level of 150 mg/l, the COD removal efficiency of the closed system (Figure 14) was greater by 15 per cent, and the solids concentration in the open system (Figure 8) was greater by 55 per cent. Again, the advantage of a closed pond is apparent even though it is not so obvious as in the previous case. When the load was increased to 250 mg/l glucose, the difference in the COD removal was only seven per cent in favor of the closed pond (Figures 15, 17), and the solids were higher in the closed system by 46 per cent. This result seems reasonable, since some of the  $\text{CO}_2$  produced by bacterial metabolism in the open pond could have escaped to the atmosphere. The possibility that increased algal solids (as evidenced by the darker green color in the closed pond) might have been the reason for the small increase in the COD removal in the closed system seems reasonable in view of the greater daily changes in

the DO in the closed system than in the open system -- 13.5 (regenerated system) and 23.5 mg/l (System I) in the closed systems in comparison with 10.4 mg/l in the open system (Figure 9). There was no significant difference in allowable loading when expressed on a volumetric basis (mg/l); however, if the loadings were expressed on an areal basis (lb/acre/day), the allowable loading for the closed pond would increase by 25 per cent, because the operating volume was 50 liters in the closed pond while in the open pond it was 40 liters; the surface area of the ponds and detention times were the same in both cases.

The open pond which received 250 mg/l glucose (Figure 9) reached zero DO levels at the end of the dark periods in spite of the fact that the maximum DO levels at the end of the light periods were well above the saturation values. Any increase in the organic loading would have resulted in creating anaerobic conditions for a considerable time; therefore, for the ponds and experimental conditions herein employed, the maximum allowable loading which can be treated aerobically in a 10-day detention period could be taken as 250 mg/l. Wu (79) used somewhat similar ponds and concluded that 300 mg/l glucose should not be exceeded in order to avoid overloading ponds operating at a detention period of ten days.

In order to evaluate the contribution by algal photosynthesis to the overall oxygen balance of the ponds, the maximum allowable loading in ponds without algae was

estimated. The open ponds were subjected to increasing loads which eventually produced anaerobic conditions. The dissolved oxygen in the effluent of the open pond which received 50 mg/l glucose (Figure 19) was 3.7 mg/l. The DO at the center of the pond was close to 3.2 mg/l; the COD removal was 70 per cent. From these data it can be seen that the pond was not loaded to its maximum capacity. When the pond was subjected to 80 mg/l of glucose (Figure 20), the average DO levels were 3, 2, and 0.3 mg/l at the effluent, center, and the influent end, respectively. The DO at the influent end varied from a maximum of 0.6 mg/l to a trace amount on the third day of operation. This indicated that the pond was operated at maximum tolerable loading for total maintenance of aerobiosis. The COD removal was 76 per cent. The open pond became anaerobic when the influent feed concentration was 120 mg/l glucose (Figure 21). Even though the DO at the effluent end was approximately one mg/l, no DO was measurable in the first reach (inflow side to center) of the pond. The average COD removal was 81 per cent. From these results it could be concluded that the physical reaeration process alone may maintain aerobiosis in a pond of this configuration when the influent contains glucose at a concentration of up to 80 mg/l.

Comparing these systems without algae with open systems with algae, the relative contribution of photosynthetic oxygenation and physical aeration can be estimated. It was

seen previously that an open pond with algae could be operated aerobically up to a loading of 250 mg/l glucose (Figure 9). It is interesting to note that the COD removal efficiency in that system (open with algae) was 76 per cent, and the open pond without algae treating 80 mg/l glucose (Figure 20) also exhibited the same efficiency of 76 per cent. Under this criterion, i.e., maintenance of aerobic conditions, the additional aeration mechanism (photosynthesis) allowed the system to accommodate a three-fold increase in organic loading. Roughly, two-thirds of the aeration could then be attributed to the photosynthetic process, and one-third to the physical aeration process. In other words, the contribution by biological oxygenation is twice that of physical aeration. However, it should be noted that the samples taken for the determination of COD in the two systems were obtained in a different manner. In the ponds containing algae, COD determinations were made on the filtered effluent, whereas the unfiltered effluents were used for the pond devoid of algae. In the pond devoid of algae, the solids production was low due to the lower loading and the small amounts of solids which were produced settled to the bottom of the tank and the effluent was clear. In brief, even though different sampling procedures were used, the effluent from the pond devoid of algae was for all practical purposes equivalent to the filtrate.

There was probably a difference in the degree of quiescence in the dark and light ponds which was caused by

alternation of light and dark periods in the lighted ponds. As may be recalled, the system containing algae was operated on a light cycle of 12-hours-on and 12-hours off. The lighting increased the temperature of the mixed liquor by approximately  $4^{\circ}\text{C}$ , and during the dark periods the temperature dropped  $4^{\circ}\text{C}$ . This alternation in the temperature could be expected to cause convection currents which would have accelerated the physical gas transfer process as well as providing better mixing of the reaction liquor. Also, evidence for "phototaxis" was observed, i.e., the algae tended to migrate toward the water surface during the light period. This movement of the population, in addition to the temperature change, tended to improve mixing, and it can be said that the pond containing algae (like ponds in the field) constitutes a semiquiescent body of water, whereas the totally dark pond might be termed quiescent. These differences (although worthy of note) seem somewhat small, and it is felt that they do not militate against the validity of the comparisons which were made between totally dark and light-dark ponds.

In field ponds, wave action created by wind (which was absent in the laboratory ponds) might exert an influence by increasing the area of the air-liquid interface and thus would enhance mixing. Under these conditions more oxygen would be absorbed during subsaturation periods, and more oxygen would be stripped during supersaturation periods compared to the laboratory ponds. Even though the light

intensity in the field would be higher than that used in the laboratory (approximately 8000 ft-c in the field versus 450 ft-c in the laboratory study), this may not be of any additional use for the photosynthetic process, since the higher intensity is well above the "saturating" light. On the contrary, while light was available to the laboratory units uniformly at all the light periods, field ponds would not get sufficient lighting on overcast days. This factor might be expected to lower the photosynthetic oxygenation. However, there is another factor which enhances the biological aeration, i.e., the depth of light penetration. In the laboratory ponds the light intensity at the surface was 450 ft-c, and at the bottom would be approximately 90 ft-c. Photosynthesis occurs in the one-foot depth of the pond at a maximum rate near the surface and at a light-limited rate near the bottom, and the variation is logarithmic. In the field ponds, assuming an average intensity of 6000 ft-c at the surface, the intensity at about 1.75 ft would be approximately 450 ft-c. Photosynthesis will occur at the maximum rate at this 1.75 ft depth. In addition, in the next one-foot depth, 1.75 to 2.75 feet, algal oxygenation will take place which alone will be equal to that of the laboratory ponds. So, in photosynthetic oxygenation also the factors encountered in field ponds are opposite in effect and tend to cancel each other; scum formations would reduce the effectiveness of both the aeration mechanisms. In view of these considerations, the relative contributions by physical



reaeration and algal oxygenation in the field ponds also could be expected to be in the ratio of 1:2. Another point to be considered in the extrapolation of the laboratory results is the greater depth of field ponds compared to the depth of the laboratory units. Since both the aeration mechanisms depend upon the surface area of the pond -- physical aeration on the air-liquid interfacial area and photosynthesis on the area of the lighted surface -- an increase in depth or in effect a decrease in surface area would reduce the overall capacity of the pond. The comparatively greater depths might not affect the relative contribution of the two aeration processes, but could lower the maximum allowable loading of the ponds. Furthermore, the operation in the laboratory was a controlled one, whereas in the field such controlled conditions do not exist.

#### Physical Gas Transfer Characteristics of the Ponds

The physical oxygenation and deoxygenation characteristics of both ponds used for continuous flow studies (Figures 22 to 25) are given in Table IV. It can be seen that the provision of baffles in one of the ponds did not change the aeration characteristics, and both the processes of deaeration and reaeration exhibited essentially the same first order velocity constant. The observed and computed saturation values were expressed as percentages of the values given in Standard Methods (corrected for temperature only); the average of those percentages was 92.61. In a

TABLE IV

## PHYSICAL DEOXYGENATION AND OXYGENATION CHARACTERISTICS OF EXPERIMENTAL PONDS

#	Characteristics	Deoxygenation		Oxygenation	
		Pond D	Pond C	Pond D	Pond C
1	Saturation value observed, mg/l	8.27	8.32	8.18	8.15
2	(1) as % of St. Method value, %	93.13	93.48	93.38	93.25
3	Saturation value, computed, mg/l	8.21	7.81	8.14	8.26
4	(3) as % of St. Method value, %	92.45	87.75	92.92	94.51
5	$K_2$ , day <sup>-1</sup> based on (1) and base 10	0.288	0.305	0.308	0.298
6	$K_2$ , hr <sup>-1</sup> based on (1) and base 10	0.0120	0.0127	0.0128	0.0124
7	$K_2$ , day <sup>-1</sup> based on (1) and base e	0.662	0.702	0.708	0.685
8	$K_2$ , hr <sup>-1</sup> based on (1) and base e	0.0276	0.0292	0.0294	0.0285
9	$K_2$ , day <sup>-1</sup> based on (3) and base 10	0.282	0.292	0.316	0.290
10	$K_2$ , hr <sup>-1</sup> based on (3) and base 10	0.0118	0.0122	0.0132	0.0121
11	$K_2$ , day <sup>-1</sup> based on (3) and base e	0.649	0.672	0.726	0.667
12	$K_2$ , hr <sup>-1</sup> based on (3) and base e	0.0271	0.0281	0.0304	0.0278

previous study in this laboratory (94) the average of such values for forty experiments was 92.06.

The rates of absorption and stripping of  $O_2$  being the same, the total quantity of gases lost or gained depends upon the magnitude of the driving force, namely, deficits or excesses, and the duration of each operation. In systems where the period of operation above saturation and below saturation are equal, the mass of  $O_2$  transferred depends only on the difference in DO concentrations. For such cases where the maximum DO levels exceed 16 mg/l, the oxygen lost to the atmosphere would be more than the gain from the atmosphere. It can be seen from Table III that all closed systems except the "regenerated" system would have experienced a loss of oxygen to the atmosphere had they not been closed, thus obviating some of the benefits of photosynthetic oxygenation.

#### Biological Deoxygenation by Organotrophs

The logarithmic oxygen uptake rates (increasing first order,  $K_1$ ,  $hr^{-1}$ ) for various loadings in both the ponds and BOD bottle systems (Figures 26 to 33) are shown in Table V. In the last column the average  $K_1$  values of duplicate experiments are given. These  $K_1$  values, individual and average, for the various substrate concentrations are plotted in Figure 42.

The range of substrate concentration employed in these studies was from 40 to 100 mg/l glucose. Referring to Table III, it is noted that the effluent COD under "balanced

TABLE V

DEOXYGENATION CONSTANTS DUE TO ORGANOTROPHS FOR  
VARIOUS SUBSTRATE CONCENTRATIONS

Glucose mg/l	System	Expt. shown in Figure #	$K_1, \text{hr}^{-1}$	Average of Duplicates
40	Pond	26	0.104)	0.108
40	Pond	27	0.113)	
40	BOD Bottle	26	0.082	0.082
60	Pond	28	0.128)	0.123
60	Pond	29	0.117)	
60	BOD Bottle	28	0.134)	0.105
60	BOD Bottle	29	0.076)	
80	Pond	30	0.162)	0.139
80	Pond	31	0.115)	
80	BOD Bottle	30	0.116)	0.109
80	BOD Bottle	31	0.102)	
100	Pond	32	0.161)	0.148
100	Pond	33	0.135)	
100	BOD Bottle	32	0.123)	0.104
100	BOD Bottle	33	0.084)	

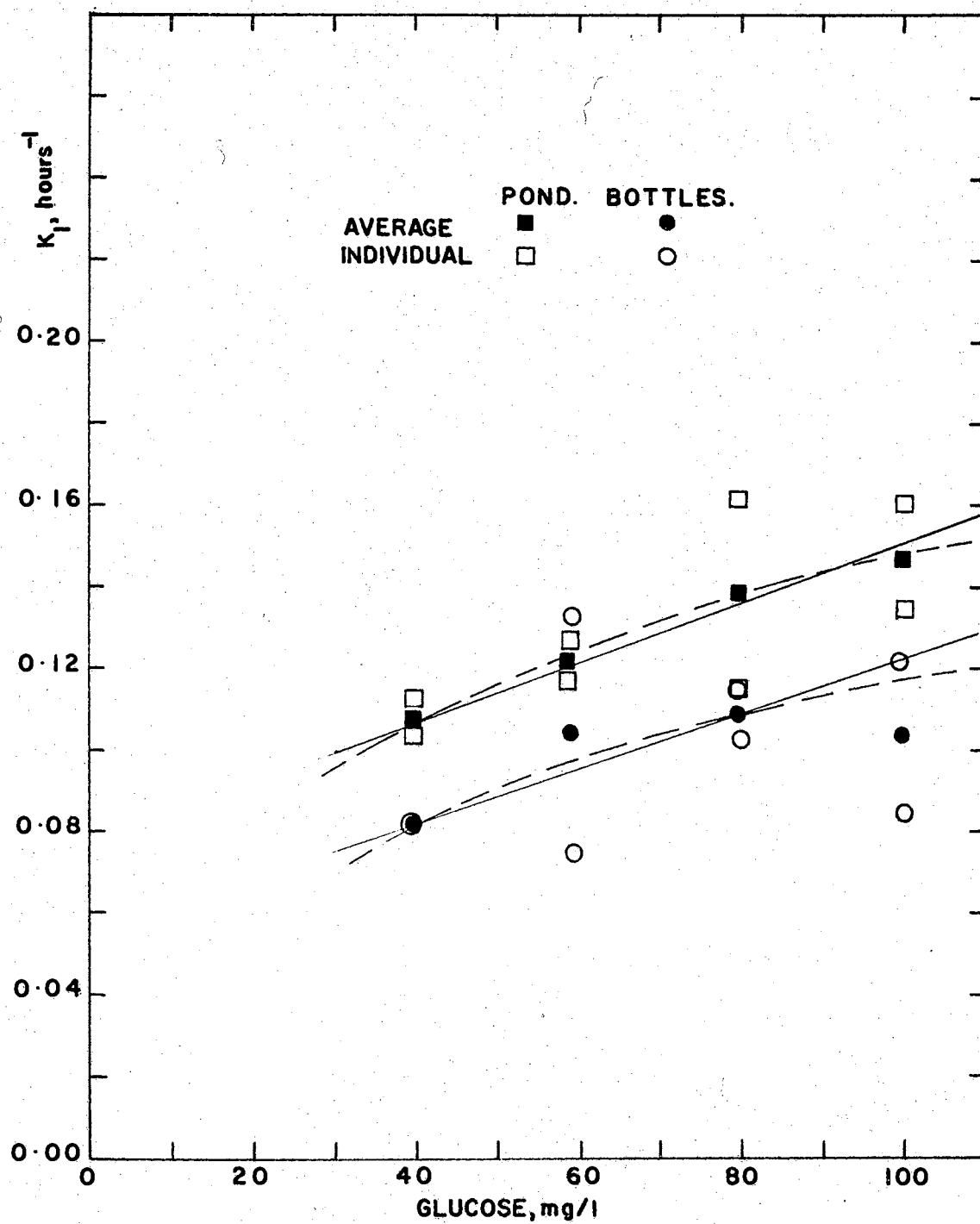


Figure 42 - Relation between substrate concentration and deoxygenation constants due to organotrophs.

operation" of the various systems, both open and closed, was in the range between 40 and 114 mg/l. Therefore, since it has been fairly well established that biological growth is dependent upon substrate concentration, the range of substrate concentrations used in the batch deoxygenation studies might be expected to yield a fairly reliable estimate of the organotrophic deoxygenation rate in the ponds. In general practice, deoxygenation studies are conducted in standard BOD bottles. The applicability of the results obtained in such fashion have been questioned by various investigators (95)(96). Gaudy, et al. (97) quoted Monod to the effect that "he was sometimes accused of feeling that if it (a certain phenomenon) happened in Escherichia coli, it also happened in elephants." To answer the somewhat analogous question "If it happens in BOD bottles should it happen in the pond?", the deoxygenation studies were conducted in the experimental ponds as well as in BOD bottles. It should be recalled that under "balanced operation" in the experimental oxidation ponds the solids concentration in the effluent was fairly constant. At a given dilution rate, steady biological solids concentration could occur only if the cells were in a logarithmic growth phase. Therefore, in these batch deoxygenation studies, increasing first order rates in the logarithmic growth phase were calculated.

As can be seen from Table V and Figure 42, the  $K_1$  values increased with increasing substrate concentration in both the pond and BOD bottle system. This indicates that

under conditions prevailing in the "balanced operation," biological deoxygenation was proportional to the substrate concentration, and even at the highest concentration the growth rate, as reflected in the  $O_2$  uptake, was substrate-limited.

From the plot of oxygen uptake rates for various substrate concentrations shown in Figure 42, it cannot be determined whether the increase in  $O_2$  uptake rate with increasing glucose concentration follows the hyperbolic relationship of Monod, since the data could also be fitted to a straight line. However, this observation cannot be taken as a disproof of the Monod relationship, because the range of substrate concentrations was very small (40 to 100 mg/l). It has been found that for heterogeneous populations, the relation between growth rate,  $\mu$ , and substrate concentration is in accord with Monod's equation and approximately 500 mg/l of substrate is required to attain the maximum growth rate (at temperatures comparable to those employed in this study). It therefore seems that the four substrate concentrations would encompass a narrow degree of curvature on a hyperbolic curve. It is generally assumed that the  $O_2$  uptake curve reflects the population curve during logarithmic growth; therefore, one might expect that the type of relationship exhibited on the basis of growth rate would also hold true for  $O_2$  uptake rate. Ramanathan (98) has shown in preliminary experiments that there is an apparent linear relationship between  $O_2$  uptake rate and

growth rate for heterogeneous populations of sewage origin grown on Glucose. Further work along this line would be useful.

The  $K_1$  rates calculated from the BOD bottle data were on the average 25 per cent lower than the rate constants calculated from the data obtained in the ponds. Nejedly (99), in his studies on the rate of  $O_2$  uptake in BOD bottles as compared to rivers, observed lower rates in the BOD bottles. He attributed this to the fact that the river comprised a turbulent system which provided better mixing which, in turn, caused a higher rate of  $O_2$  uptake. This explanation is not applicable to the present study, because both the ponds and the BOD bottles were maintained under quiescent conditions. It is difficult to envision how the mere difference in volume of reaction fluid could affect the results, but enough data were obtained to indicate that a real difference did exist in  $O_2$  uptake rate, and this aspect could prove to be a fruitful channel for further investigation.

#### Biological Deoxygenation by Photo-autotrophs

The results of deoxygenation studies using photo-autotrophs were presented in Figures 34 to 41. Glucose at a concentration of 500 mg/l was used as substrate in all heterotrophic experiments, since it was felt that at this concentration growth would not be substrate-limited (or at any rate, severely limited). Gaudy and co-workers (100)(101) have proved conclusively that the mechanisms as well as the



kinetic order of substrate removal were different when low and higher initial bacterial solids were employed in laboratory scale activated sludge process, and they showed that less oxygen was consumed at the point of substrate removal with high initial solids concentrations.

Pearsall and Bengry (39) cultured Chlorella in the dark on glucose and found that the growth rate was at first exponential; then as the cell number reached approximately 600 cells/cu mm ( $6 \times 10^6$  cell/ml), the growth curve became linear. The linear phase did not depict the endogenous phase, since only 20 per cent of the substrate was used even after six days of linear growth. To determine whether a difference in the kinetics of oxygen uptake existed at different initial algal concentrations and to determine the range of concentrations at which the two different modes of oxygen uptake exist, experiments were conducted with varying initial algal concentrations from 113 mg/l to 896 mg/l.

As can be seen from the figures (34 to 38), the  $O_2$  uptake curve of the system with an initial algal concentration of 896 mg/l (Figure 34) was linear during the active substrate removal period, whereas in the systems with initial algal concentrations of 215 (Figure 37) and 113 mg/l (Figure 38) the oxygen uptake curves were more characteristic of logarithmic growth. The systems with 448 (Figure 35) and 400 mg/l (Figure 36) algae showed kinetics which suggested that logarithmic growth phase was approached

during the active substrate removal period.

The kinetic order and the rate constants for all of the experiments are summarized in Table VI. Also shown are the percentages of the theoretical oxygen demand exerted at the time of substrate removal (for both glucose and total COD). Before discussing the relation between the values shown in Table VI for Chlorella pyrenoidosa and the findings of other workers for heterogeneous microbial populations, it is appropriate to discuss the validity of the results from which the information for the table was obtained.

In an actively growing heterogeneous microbial population, the substrate utilized by the organisms is channeled into synthesis of new cell mass and into respiration ( $O_2$  uptake) for the production of energy for various metabolic activities. All of the substrate removed can be reasonably accounted for as the sum of cell synthesis and respiration. Gaudy, et al. (102) have reported four methods by which a material balance can be made, and they calculated percentage recovery close to 100 per cent for their experimental data. To the author's knowledge, this type of balance has not yet been applied to algal cultures grown heterotrophically.

A materials balance for the present study was made (on the basis of carbon) (102) using the COD removed, solids produced, and the oxygen uptake at the point of substrate removal. In all of the systems the percent recovery was only  $66 \pm 6$  per cent. While no proven reason for this

TABLE VI  
KINETIC CONSTANTS AND PERCENT THEORETICAL OXYGEN  
UPTAKE FOR SYSTEMS WITH VARIOUS INITIAL  
SOLIDS CONCENTRATIONS

Initial Solids mg/l	Figure #	Kinetic Order	First Order hr <sup>-1</sup>	Zero Order mg/l O <sub>2</sub> /hr	Percent Theoretical Oxygen Demand Exert- ed at the Time of	
					COD Removal	Glucose Removal
896	34	0	-	15.73	17.3	17.0
448	35	0	-	9.15	19.5	20.0
400	36	0*	-	7.29	18.0	17.9
215	37	1	0.089	-	18.8	26.3
113	38	1	0.095	-	19.8	26.7

\* Approaching First Order

apparent low recovery can be advanced, the following explanation seems to present a reasonable possibility for the observed discrepancy. The method for the determination of COD is based upon the assumption that all organic materials in the sample are oxidized under the conditions of the test. While this assumption is true for many organic substances, there are some organics (e.g., aromatic hydrocarbons) which are not registered by the COD test (87). If such organic substances were produced by the algae and were present at the point of indicated substrate removal, the indicated COD would not provide a means of estimating the actual concentration of the remaining carbon resource. Thus a greater removal of carbon source than actually occurred would be registered and the percentage recovery would be low.

The carbon balances for the systems at various times after the completion of the substrate removal were also calculated; the percentage recovery increased as the run progressed. On the average, the maximum percent recovery was 75.5 per cent, approximately 10 per cent more than the values obtained at the point of substrate removal:

Initial Solids mg/l	Percent Recovery at the Time of COD Removal	Maximum Percent Recovery near End of Run
896	67.5	81.5
448	63.8	79.6
400	60.3	64.2
215	64.9	73.2
113	72.0	78.8

The improvement in the balance was due to a significant amount of oxygen uptake after the apparent exhaustion of the carbon source, as indicated by the COD test. The increased  $O_2$  uptake could be attributed to endogenous uptake or to the utilization of carbon source not registered by the COD test. However, the major part of the increased  $O_2$  uptake can be attributed to the latter cause, since the endogenous  $O_2$  uptake was lower than the increased demand after the exhaustion of COD.

The percentages of theoretical oxygen demand exerted at the time of glucose removal (as measured by the anthrone test) at the time of COD removal are given in Table VI. It can be seen from Table VI that the percent theoretical  $O_2$  demand exerted at the point of substrate removal, whether expressed as COD or glucose, increased as the initial solids concentration decreased. The decrease is more pronounced when the analysis is based on the anthrone test. The difference in the decrease of percent theoretical oxygen demand using the glucose and total COD removal supports the premise made previously that some nonregisterable COD, probably produced during substrate removal, existed in the system. The limited (three solids concentrations) investigation of McWhorter and Heukelekian (103) and the exhaustive work by Gaudy and co-workers (100)(101) with bacterial populations showed the same phenomenon of decreased percent theoretical oxygen demand with increasing initial solids concentration at the point of COD removal. Comparing

values obtained by McWhorter and Heukelekian with those obtained in this research, it can be noted that there was a small difference of four per cent at the higher levels of solids (21 per cent at 1010 mg/l [103] and 17.3 per cent at 906 mg/l algae). This difference increased to 10 per cent at low solids levels (30 per cent at 0.5 per cent settled sewage seed [103] and 19.8 per cent at 113 mg/l algae). These differences can be expected in the light of the conclusion of Rao and Gaudy (100) that  $O_2$  utilization, although dependent upon the initial concentration of biological solids, can vary with each specific sludge.

Concerning the percentage theoretical  $O_2$  demand exerted at the point of glucose (anthrone) removal, the data of this research do not support the conclusion of McWhorter and Heukelekian that a constant 18 per cent was exerted. The decreasing trend in percent theoretical oxygen demand at the time of glucose (anthrone) removal observed in the present investigation confirms the findings of Rao and Gaudy. In addition, the values obtained are in the same range as their values. In Figure 43 the values obtained in this investigation are plotted along with the values obtained by Rao and Gaudy (see Figure 8 [100]). It seems reasonable to conclude that, under heterotrophic metabolic conditions, Chlorella pyrenoidosa exerted percentage theoretical oxygen demands at the point of substrate removal which were inversely proportional to the initial solids concentrations in the range tested. Thus, for the

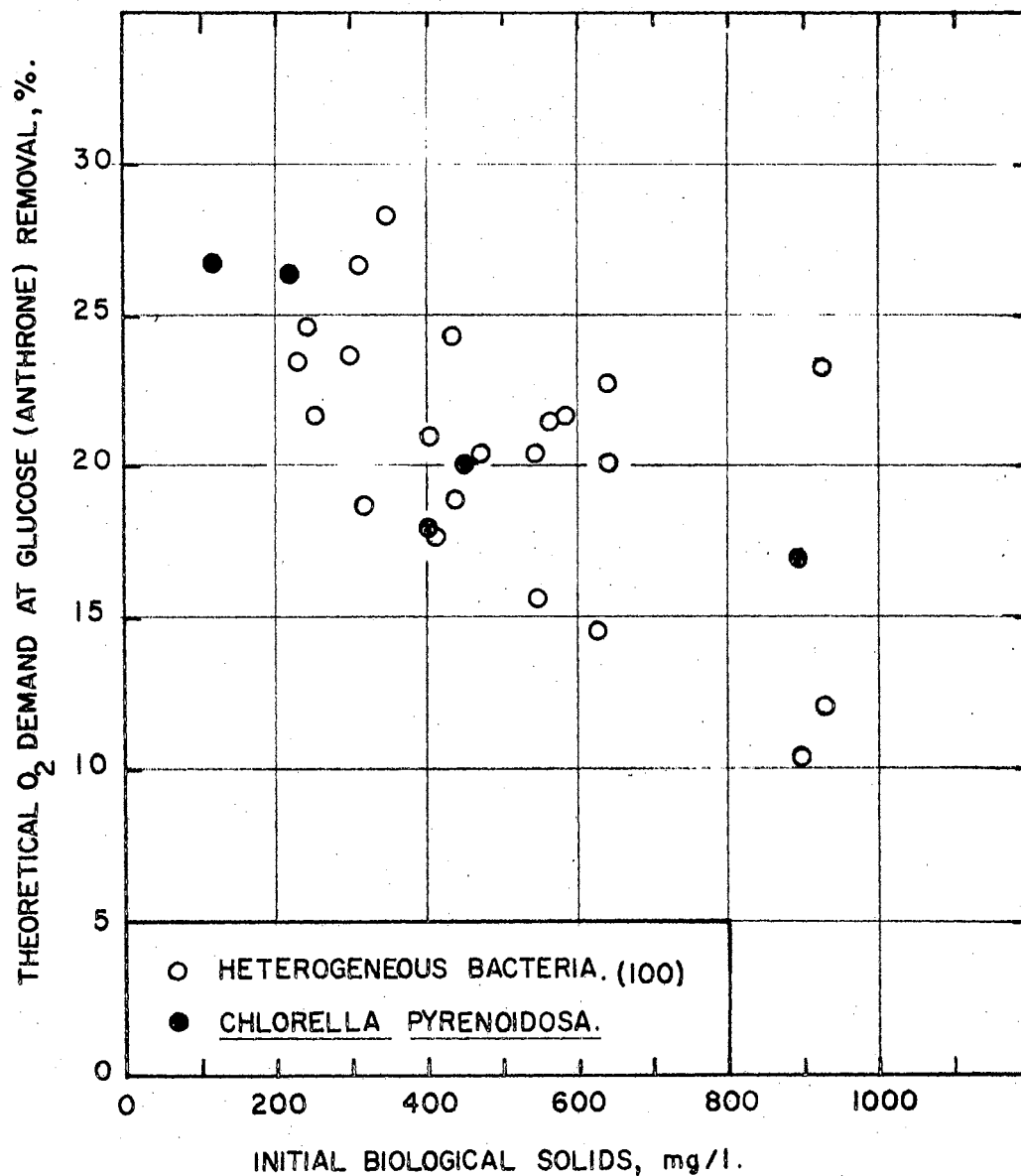


Figure 43 - Effect of initial biological solids on percentage of theoretical oxygen demand exerted at time of glucose (anthrone) removal.

algae as well as bacteria it would appear that the initial biological solids concentration affect both the kinetics of the system (zero order at high solids, first order at low solids) and the amount of oxygen required for removal of the original exogenous carbon source. Also, it would appear that the point at which the observable shift in kinetic mode occurs can be defined by the food/micro-organisms ratio and the value is approximately unity.



## CHAPTER VII

### SUMMARY AND CONCLUSIONS

It was seen that the experimental oxidation ponds could be operated under "balanced conditions" wherein parameters such as maximum DO, minimum DO, biological solids, and effluent COD were (more or less) constant. In some systems there were two such periods of "balanced conditions" with respect to the daily DO changes (closed systems at 100 mg/l and 250 mg/l), and even in these cases the biological solids and the effluent COD were approximately constant. At times the "balanced operation" was disturbed temporarily (closed pond at 150 mg/l) and in two cases disrupted permanently (open pond at 250 mg/l, closed pond at 250 mg/l, system I). In some cases the algal activity, and hence the daily DO change, was less at the beginning of the operation of the ponds.

The data indicated that atmospheric  $\text{CO}_2$  was fixed by the algal population of the open pond and that this effect made the open pond at the low loading (100 mg/l) into a polluting pond rather than a waste treating pond, since approximately 100 per cent more carbon was present in the effluent than was present in the influent. In the 150 mg/l system the carbon content was increased by approximately

25 per cent, whereas there was a 50 per cent decrease in carbon content in the 250 mg/l system. Because of the gain in carbon from the atmosphere, closing the ponds to the atmosphere increased significantly the purification efficiency at low loadings, and moderately at higher loadings. Though the provision of gas-impermeable, light-transmitting covers for field ponds (which would prevent entry of  $\text{CO}_2$  and escape of oxygen) is not impossible from an engineering point of view, it should be thoroughly investigated before such a recommendation is made. Since more atmospheric  $\text{O}_2$  would be expected to enter the pond liquor near the effluent end of the pond, it seems apparent that long detention times should not significantly improve carbon removal; indeed, they may tend to obviate the purpose for which the pond was installed. Oxidation ponds are not designed (at any rate not at present) as reactors for growing algae, but as reactors for removing organic matter in waste waters.

The data indicated that the maximum allowable loading in an open pond without algae was 80 mg/l glucose, whereas the load could be increased to approximately 250 mg/l glucose with an algal population operated with the same detention period. Thus it could be concluded that the net contribution from photosynthesis was approximately twice that from oxygenation by physical transfer from the atmosphere. The maximum allowable loading (for maintenance of aerobic conditions) of 250 mg/l in the open ponds, with algae, if expressed as an areal loading amounted to 63 lb COD/acre/day.

Wu (79) used a 36-liter pond with 30 liters of waste liquor and a surface area of  $1387 \text{ cm}^2$  compared to the 40-liter volume and  $1500 \text{ cm}^2$  pond in the present study, and showed that 61.7 lb COD/acre/day could be satisfactorily treated.

In the last subsection of the literature review it was pointed out that the substrate removal efficiencies of oxidation ponds are expressed by two means--percent COD removal and percent BOD removal--and the percent COD removal was always lower than the percent BOD removal. Determination of the purification efficiency in field ponds (74)(75)(76) have been made on both bases. The COD removal efficiencies attained in the present research when the ponds were loaded to their maximum capacity to maintain aerobic conditions were 76 per cent in the open system and 83 per cent in the closed system. If these organic loading removal efficiencies had been expressed on a BOD basis, they would be well over 90 per cent. Therefore, the purification efficiency (for filtrate) of the laboratory pond approximated the field pond efficiencies based on pond effluents. Various factors which come into play when laboratory data are extrapolated to the field conditions were pointed out previously, and it was seen how these factors tended to counterbalance one another, thus indicating that the relative contributions of the two aeration processes (physical transfer and photosynthetic oxygenation) found in the present laboratory studies could also be expected to exist

in field ponds.

In general, oxidation ponds seem to present a "paradox." They are attractive in certain localities because of their low cost of construction and low operation and maintenance costs. If low loadings are employed in order to maintain aerobic conditions, the photo-autotrophic organisms fix atmospheric carbon dioxide, thereby militating against the aim of waste water treatment. The situation is so severe at low loadings that even when solids separation is not taken into consideration, oxidation ponds add more pollution (organic carbon) than they remove. To avoid this undesirable aspect, oxidation ponds should be operated at near maximum loadings, but if they were operated in such a manner they would tend to become anaerobic when any possible shock loads were added to the system.

### CONCLUSIONS

Based on the results and the author's interpretation of them, the following conclusions seem warranted:

1. Oxidation ponds operated at low loadings with a detention period of ten days are not really in balance biologically, because bacteria do not supply all of the required carbon for the algae, and this leads to a case where the effluent can contain more carbon than the influent.
2. In general, due to physical oxygen exchange processes across the air-liquid interface of the

pond, more oxygen is lost to the atmosphere than is gained from it.

3. The adverse operational aspects brought out in conclusions 1 and 2 may be eliminated by closing the ponds to the atmosphere without shielding light, i.e., use of transparent, gas-impermeable cover. For example, a "Saran"-type membrane attached to a floating grid could be tried.

4. Closed ponds increased the purification efficiency significantly at lower organic loadings, and moderately at higher loadings.

5. For satisfactory performance on the basis of maintenance of aerobic conditions, the maximum allowable loading in an open pond was 250 mg/l glucose at 10-day detention periods or 63 lb COD/acre/day.

6. An open pond devoid of algae in which physical reaeration was the only aeration mechanism responded satisfactorily to an organic loading of 80 mg/l glucose.

7. Based upon the laboratory studies, it seems reasonable to predict that in field installations photosynthetic oxygenation contributes twice as much to the oxygen resource of the pond as does physical aeration.

8. The similarity of the results obtained by Rao and Gaudy (100) using organotrophic mixed microbial populations and those in the present study using Chlorella pyrenoidosa under organotrophic growth conditions suggests that biological solids concentration exerts a general effect upon the kinetics and mechanisms of heterotrophic metabolism.

## CHAPTER VIII

### SUGGESTIONS FOR FUTURE WORK

Analysis of the results of this investigation suggested the following items for possible future investigation:

1. Experimentation with a closed pond in a field scale pilot plant might be usefully undertaken. The suitability of various transparent membranous materials and means of holding them in place should be explored.

2. Further investigations pertaining to the relation between organic removal efficiency and detention period seem warranted, especially for detention periods of less than ten days.

3. The effect of sludge deposition on the oxygen resource of oxidation ponds needs further study.

4. If further laboratory work using the experimental ponds is undertaken, the work should include the following aspects:

- (a) Separate determination of algal and bacterial population in the ponds.

- (b) Determination of complete carbon balance, i.e., organic carbon, free  $\text{CO}_2$ , carbonates in the influent and effluent, and the carbon component of the biological solids.

(c) Correlation of BOD and COD on influent and effluent samples.

(d) Determination of the major organic compounds in the effluent (filtrate).

5. Further work to confirm the effect of initial algal concentration on the kinetics and mechanism of substrate removal during heterotrophic metabolism which was observed in the present study would be of considerable value.

(Further experimentation along these lines has already been designed.)



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